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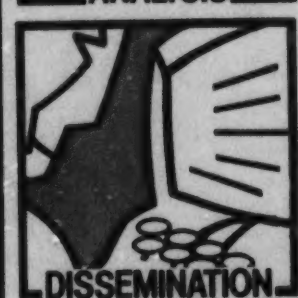

SPECIAL LISTING

CURRENT CANCER RESEARCH

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MODIFICATION OF CHEMICAL CARCINOGENESIS IN MODEL SYSTEMS

OCTOBER 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CURRENT CANCER RESEARCH

I. ENHANCEMENT OF CHEMICAL CARCINOGENESIS IN MODEL SYSTEMS

A. ENHANCEMENT OF EFFECTS OF SPECIFIC CHEMICAL CARCINOGENS BY OTHER CHEMICAL AGENTS

1. HEAVY METALS, POLYCYCLIC HYDROCARBONS AND HORMONAL INTERACTIONS IN CELL CULTURE (ABBREV)

de Vellis, J. S., Harary, I., Herschman, H., University of California, Los Angeles Campus, School of Medicine, Dept. of Anatomy, *2025 Zonal Ave., Los Angeles, California,* 90024, United States of America

We are investigating the direct effects of heavy metals and polycyclic hydrocarbons on brain and muscle functions by using differentiated cell cultures of the three main brain cell types (neurons, astrocytes and oligodendrocytes) and of cardiac and skeletal muscle cells. We will investigate the molecular mechanisms involved in each cell type in order to establish a molecular basis for possible sensitivity, development of tolerance and resistance to these agents. The potential interactions between heavy metals, endogenous mitogens and hydrocarbons in the overall oncogenic process will be studied in cultures and in the animal. Heavy metals will be tested in culture systems for direct as well as co-carcinogenic effects. We also wish to determine the mechanisms by which cells accumulate the metal binding protein metallothionein in response to high levels of Cd ion and Zn ion in their environment. We hope to elucidate the role of de novo protein synthesis, de novo mRNA synthesis, protein turnover, mRNA turnover, protein stabilization, mRNA stabilization and hormonal status in this cellular response to an environmental challenge by heavy metals.

This continuing project has already resulted in the publication of several papers. A five-year report will be submitted to EPA during the summer of 1980.

We have developed and validated primary and clonal cell cultures of rat liver, brain, and skeletal and cardiac muscle as fast, easy, quantitative and reliable test systems for screening of toxic agents. Acute and chronic exposure of cultured cells to lead resulted in a selective inhibition of the expression of differentiated functions. Cadmium and zinc induced metallothionein, a metal-binding protein, in a rat liver cell line grown in a chemically defined medium. The induction requires RNA and protein synthesis. Metallothionein was also induced in a human cell line, HeLa. Glucocorticoid augments the metal induction of metallothionein. These studies will allow us to answer this fundamental question of the role of steroid hormones in the modulation of the detoxification response.

2. CO-CARCINOGENICITY OF FOUNDRY PARTICULATES - NCI

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The co-carcinogenic effects of particulates in the intratracheal instillation model for lung co-carcinogenesis are well known. Ferric oxide (Saffiotti, 1979), carbon (Farrell et al., 1972), and magnesium oxide (Stanback et al., 1975), in combination with the ubiquitous carcinogen, benzo(a)pyrene (B(a))) significantly increases the number of pulmonary tumors as compared to B(a)P alone. The results obtained with aluminum oxide (Farrell et al., 1972) are questionable.

NIOSH estimates that there are more than 4,400 American foundries employing 200,000 workers. Recent epidemiological studies reviewed by Egan and coworkers (1977) have concluded that there is a significant excess of lung cancer, as well as nonmalignant respiratory disease, among foundry workers. Bates and coworkers (1974-1976) and others have identified polynuclear aromatic hydrocarbons as contaminants in the foundry environs.

This project will evaluate the effects of various particulates used in the foundry process in conjunction with intratracheal administration of B(a)P. Some of these particulates are currently in use as substitutes for silica sand because of the vast silicosis problem associated with this material in foundry workers. However, virtually nothing is known about their co-carcinogenic or fibrogenic properties. The particulates that will be studied are: zirconium silicate, silica sand, olivine, chromite sand, aluminum silicates, Min-U-Sil (reference quartz), and ferric oxide. Saffiotti's model of administration will be used for 50 Syrian golden male hamsters. All particulates will be given alone and in combination with B(a)P. Pulmonary pathology will be emphasized. This study is being conducted by contract (210-79-0036) which commenced in September 1979.

3. CO-CARCINOGENICITY OF ENVIRONMENTAL METALS AND MINERALS WITH SYSTEMIC CARCINOGENS IN THE RAT LUNG (ABBREV)

Heath, J. C., Strangeways Research Lab., Dept. of Cancer Research, *2 Worts Cswy., Cambridge CB1 4RN, England, United Kingdom*

OBJECTIVE: To investigate the carcinogenic action of very small lung deposits of mixed particulate minerals and metals of environmental importance, acting alone or together with a very low level of a systemic carcinogen.

APPROACH: The particulate minerals and metals are administered in a small amount by a single intratracheal instillation under anaesthesia. After about 6 weeks, when the pulmonary lesion is well established, a short course of once-weekly subcutaneous injections of a very low dose of a systemic carcinogen such as a nitrosamine is given. No further treatment is given, and the animals are allowed to live out their lives or to live until increasing respiratory distress renders their destruction desirable. The lungs and other relevant internal organs of all rats are fully examined postmortem.

PROGRESS: Lung lesions induced by minimal deposits of particulate silica or fibrous asbestos only very occasionally proceed to malignant metastasizing lung tumors. With the concurrent action of particulate cobalt, nickel or cadmium metals at 10% w/w of the total lung particulate deposit, rather more malignant lung tumors are produced. The further administration of a very low level of a systemic carcinogen such as a nitrosamine, acting systemically but at a level low enough not to induce a significant number of malignant lung tumors by itself, can increase significantly the incidence of such tumors.

4. THE METABOLIC ACTIVATION OF POLYCYCLIC HYDROCARBONS IN CARCINOGENESIS - ASBESTOS-ENHANCED CARCINOGENESIS

O'Brien, P. J., Memorial University of Newfoundland, Dept. of Biochemistry, *Health Science Complex, St. Johns, Newfoundland,* A1C 5S7, Canada

OBJECTIVE: The proposed study is primarily concerned with the mechanism of tumor induction in the respiratory tract by polycyclic hydrocarbons or by asbestos. However, the re-

sults may prove to be of general application in understanding mechanisms for the metabolic activation of carcinogens.

APPROACH: a) The mechanism of the in vivo protection by dietary antioxidants against carcinogenesis induced by polycyclic hydrocarbons is to be investigated. b) Research that is relevant for developing a genetic susceptibility test for cancer is also proposed. Such a test could be useful in minimizing possible carcinogen exposure for high risk individuals (e.g., occupation). c) Possible physiological methods will be investigated for forming singlet oxygen as a cytotoxic agent for tumor cells which could prove useful in cancer therapy. d) Finally it is intended to examine whether asbestos slows the pulmonary clearance and detoxification of polycyclic hydrocarbons and enhances hydroxyl radical formation during their metabolic activation.

5. COCARCINOGENIC MECHANISMS OF ASBESTOS

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An association has been established between environmental and occupational exposure to asbestos and the occurrence of neoplasms of the respiratory tract. The incidence of bronchogenic carcinoma in asbestos workers who smoke is increased significantly as compared to non-smokers. At present, the biologic mechanisms by which asbestos acts as a cocarcinogen are unclear. Experiments in this laboratory suggest that asbestos serves as a 'carrier' of polycyclic hydrocarbons (PCH) into the cell. In addition, asbestos induces hyperplasia and squamous metaplasia of the respiratory tract basal cells, an effect intrinsic to carcinogenesis.

This study is designed to answer the following questions: 1) Does the binding (or absorption) of PCH by asbestos relate to the structure, chemical composition and surface charge of the fiber, and does this affinity correlate with the carcinogenic potential of the asbestos? 2) What is the mechanism whereby asbestos increases the uptake of PCH by the cell and what are the structural and biochemical events in the cell that result in carcinogenic transformation? 3) Does asbestos act as a classical 'tumor promoter' (i.e., does it induce nucleic acid and protein synthesis and the activity of certain characteristic enzyme systems)? Studies will be designed to assess comparatively the effects of sized preparations of crocidolite and chrysotile asbestos in assays using tracheal organ cultures, grafts of trachea in syngeneic animals and monolayers of tracheal epithelial cells.

6. COAL LIQUEFACTION - CARCINOGENESIS MOUSE SKIN

Slaga, T. J., U.S. Dept. of Energy, Oak Ridge National Lab., Biology Div., *P.O. Box X, Oak Ridge, Tennessee*, 37830, United States of America

In view of DOE's responsibilities in developing fossil fuels for energy we will be confronted with many biological hazards from polycyclic aromatic hydrocarbons (PAH) which are associated with their conversion and utilization. These PAH may have carcinogenic, tumor initiating or promoting activities in man. We propose to study the mechanism of action of certain PAH carcinogens and tumor promoters in epidermal cells both in vivo and in vitro. The tumor initiating and promoting abilities of benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene and their cellular metabolites are being investigated in order to better define complete carcinogenesis by PAH. We have found that (-trans)benzo(a)-pyrene-7,8-diol-9,10-epoxide is the ultimate tumor initiating form of benzo(a)pyrene. However, this intermediate is not a complete carcinogen; consequently, we are determining which metabolite(s) are responsible for the tumor promoting activity. Since the majority of PAH have only been tested for complete carcinogenic activity, it is imperative that they be tested for both tumor initiating and tumor promoting activity in our sensitive mice. Studies will be expanded on the anti-carcinogenic effects of steroids, benzoflavones, antioxidants and vitamin A analogs, which may lead to a rational approach to chemoprophylaxis of environmental carcinogens. Some of these agents may also be useful in counteracting the harmful effects of accidental exposure to coal conversion products.

BIBLIOGRAPHIC REFERENCES: Slaga, T.J., et al, Carcinogenicity and Mutagenicity of Benz(a)anthracene Diols and Diol-epoxides , Cancer Research 38: 1699-1704 (1978). Slaga, T.J., et al, Dibenz(a,c)anthracene, a Potent Inhibitor of 7,12-Dimethylbenz(a)anthracene Skin Tumor Initiation, Res. Commun. Chem. Path. and Pharm. 19: 477-483 (1978).

7. EVALUATION OF THE TUMORIGENIC ENHANCING POTENTIAL IN LIVER OF POLYCYCLIC AROMATIC HYDROCARBONS

Peraino, C., U.S. Dept. of Energy, Argonne National Lab., *9700 S. Cass Ave., Argonne, Illinois*, 60439, United States of America

Studies in this laboratory have shown that dietary phenobarbital enhances hepatic tumorigenesis in rats previously fed 2-acetylaminofluorene (AAF). This sequential treatment procedure has proven useful for examining the ability of a variety of agents to act as tumorigenic enhancers in liver. The present study is designed to examine the possibility that this procedure might be useful as a means of screening energy-related pollutants (namely, polycyclic hydrocarbons) for such enhancing activity. The hydrocarbon chosen for the initial study is benz(a)anthracene, a compound with little or no intrinsic tumorigenic activity. Preliminary experiments showed that benz(a)anthracene resembles phenobarbital in its stimulatory effect on liver DNA synthesis and liver growth. When benz(a)anthracene was fed at a dietary concentration of 0.02%, following AAF feeding, no tumorigenic enhancement was observed. Subsequent experiments will involve the feeding of higher levels of benz(a)anthracene so that the enhancing ability of the hydrocarbon can be unequivocably evaluated.

BIBLIOGRAPHIC REFERENCES: C., Peraino, R.J.M. Fry and D.D. Grube, Drug-Induced Enhancement of Hepatic Tumorigenesis, pp. 421-432 in Carcinogenesis, Volume 2: Mechanism of Tumor Promotion and Cocarcinogenesis, Slaga, Sivak and Boutwell (eds.), Raven Press, NY, 1978.

8. MIXED INDUCERS AS COCARCINOGENS

Safe, S. H., University of Guelph, Dept. of Chemistry, *Guelph, Ontario*, N1G 2W1, Canada

OBJECTIVES: Halogenated aromatic chemical pollutants are potent inducers of the microsomal monooxygenase enzymes which play a pivotal role in the metabolic activation of procarcinogens to carcinogens. Thus inducers of this enzyme system may act as cocarcinogens. The effects of a number of mixed-type inducers (polychlorinated biphenyls) are being evaluated using mammalian cells (human skin fibroblasts and rat hepatocyte suspensions) in several short-term in vitro assay systems.

APPROACH: The approach used in this study focuses on two different in vitro systems: a) A two-compartment model using human skin fibroblasts as host, induced rat S-9 as a source of metabolic activation, benzo(a)pyrene and aflatoxin as the procarcinogens and unscheduled DNA repair (UDS) as an end point. b) A one-compartment model using freshly prepared hepatocyte suspensions from induced rats as the host, benzo(a)pyrene and aflatoxin as the procarcinogen and UDS as the end point.

9. THE MECHANISMS OF EPIDERMAL TUMOR PROMOTION IN HAMSTER CHEEK POUCH

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We have determined the approximate optimum condition for tumor initiation and promotion by topical application to be: initiation with 0.2% 7,12-dimethylbenz(a)anthracene (DMBA) in DMSO on alternate days for 2 weeks followed by promotion with 0.05 M retinyl acetate (RA) in DMSO on alternate days for 4 weeks. Attempts to further reduce experimental time periods by doubling dosage frequency keeping total dose constant caused intolerable cytotoxicity in the case of DMBA and too short a promotion period for acceptable tumor yield in the case of RA. Investigations of the mechanism of promotion of benign hyperplastic lesions (BHLs) are continuing. We had found that Ca influx causes promotion of BHLs. It is known that gene activation and cell differentiation is governed by intracellular K/Na ratio in the transition from larval to adult form in insects. It is also known that Ca influx induces K outflux in erythrocytes. We are therefore exploring the possibility that Ca influx lowers intracellular K/Na ratio thus giving rise to a less differentiated epithelial phenotype. This effect might become 'locked in' in initiated cells which would form benign tumor clones (BHLs). We have tested two agents of diverse nature which are

known to reduce intracellular Na/K ratio for their ability to promote BHLs: valinomycin, a K ionophore which increases K outflux and ouabain, a specific inhibitor of (Na,K)ATPase which inhibits Na efflux. Interestingly, both agents promoted BHLs when applied at 10 to the minus 5th power M under the standard protocol. Also, 0.1 M KCl markedly inhibited the promoting effect of 0.1 M Ca as would be expected if the proposed mechanism is correct. We are contemplating experiments to determine whether the various agents do indeed alter intracellular K/Na ratio in isolated pouch epithelium in vitro.

10. RETINOIDS EHNANCE MITOGENESIS BY TUMOR PROMOTERS AND POLYPEPTIDE GROWTH FACTORS

Dicker, P., Rozengurt, E., Imperial Cancer Research Fund, Membrane Physiology & Growth Regulation Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

Vitamin A derivatives (retinoids) have been reported to inhibit cell proliferation and antagonize the biological activity of 12-0-tetradecanoyl-phorbol-13-acetate (TPA), a potent tumor-promoting agent in several experimental systems. We found that retinoids at low concentrations strikingly enhance the growth-promoting activity of polypeptides like epidermal growth factor, insulin, vasopressin and fibroblast-derived growth factor and of TPA. Retinoids acting synergistically with these growth factors enhance both DNA synthesis and cell proliferation in quiescent cultures of mouse 3T3 cells and of hamster Nil-8 cells. These findings contrast with previous reports showing growth inhibitory properties of retinoids. Previous investigations were, however, performed on growing rather than quiescent cells as were used in our study. Also, previous investigations showed inhibitory effects of retinoic acid at much higher concentrations than those used by us. Possibly, retinoic acid was exerting a non-specific cytotoxic effect at high concentrations. Our findings suggest that the general use of retinoids in human neoplasia might require further critical exploration.

11. CHLORINATED WATER AND MEMBRANE FUNCTIONS AND NEOPLASIA

Sun, A. Y., Vogt, C. R., Sun, G. Y., University of Missouri, Columbia Campus, Sinclair Comparative Medicine Research Farm, *Rte. 3, Columbia, Missouri*, 65201, United States of America

Chlorine is suspected to be a cancer risk because it interacts with organic matter in water and in biological systems leading to the formation of various toxic chloroderivatives. The overall objective of this proposed study is to investigate the interaction of chlorine and/or chlorinated compounds with the cellular constituents under the physiological condition in relation to the biochemical mechanism of tumor formation as induced by this interaction. Specifically, we shall: (1) study the effect of acute and chronic treatment of chlorinated water on the structure and function of biological membranes, (2) evaluate synergism and promotion in tumorigenesis when chlorine water ingestion is combined with other chemical carcinogens and (3) examine the inhibitory effect of dietary antioxidants on the chemical carcinogenesis as induced by chlorine and dimethylnitrosamine (DMN).

The change in the compositions (lipids and proteins) and functions (membrane-bound enzymes, transport activities) of erythrocyte membranes, kidney and liver mitochondria and synaptic plasma membranes will be studied and compared in control and experiment animals. The growth rate, tumor incidence and associated histological and membrane change with either chlorine or combined with DMN administration will be examined. In addition, the inhibitory effect of dietary vitamin E and selenium on the chemical carcinogenesis as induced by chlorine alone or in combination with DMN will be evaluated. It is anticipated that the result of this investigation will provide more information about the prevalence of mutagenic agents in drinking water and in the biological system. Possible preventive measures to the chemical carcinogenesis may be developed as a result of this study.

BIBLIOGRAPHIC REFERENCES: Vogt, C.R., Liao, J.C. and Sun, A.Y. (1980) Extraction and determination of chloroform in rat blood and tissue by gas-chromatography-electron-capture detection: distribution of chloroform in the animal body. Clin. Chem. 26:66-68. Vogt, C.R., Liao, J.C., Sun, G.Y. and Sun, A.Y. (1980) Chlorination in vivo and its effect on membrane function. Trace Substances in Environmental Health 13:453-460.

12. EFFECTS OF NICOTINE ON THE CARCINOGENIC ACTIVITY OF SMOKE CONDENSATE FROM EXPERIMENTAL CIGARETTES

Tso, T. C., Bock, F. G., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

OBJECTIVE: Determine whether nicotine is a cocarcinogen in tobacco smoke. This information is essential for deciding the level of nicotine in leaf tobacco with minimum risk to the consumer.

APPROACH: Using both Bright and Burley tobacco types with low nicotine content (0.2-0.4%) add different levels of pure nicotine to establish three different levels of total nicotine and reconstitute the experimental materials into sheets, make experimental cigarettes for short-term bioassay using sebaceous gland suppression method. This may lead to long-term skin painting studies if necessary. Conduct long term mouse skin painting to test carcinogenic activity.

PROGRESS: Short term sebaceous gland suppression bioassay started on six experimental cigarette samples including Bright cigarettes (50% reconstituted and 50% low-nicotine leaf) with three nicotine levels (0.26, 1.00 and 1.68%), and Burley cigarettes (50% reconstituted and 50% low-nicotine leaf) with three nicotine levels (0.35, 0.93, and 1.22%). Burley cigarettes appeared to generate comparatively lower tar than Bright ones.

13. INDIVIDUAL GASEOUS COMPONENTS OF DIESEL EXHAUST - ASSESS ONCOGENICITY AND TOXICITY OF CONCOMITANT EXPOSURE TO CARBON PARTICULATE AND PAH

Orthoefer, J. G., Pepelko, W. E., U.S. Environmental Protection Agency, Office of Research & Development, Health Effects Research Lab., *5555 Ridge Ave., Cincinnati, Ohio*, 45268, United States of America

OBJECTIVE: To determine if prior adsorption to an organic particulate results in an increased toxicity and/or carcinogenicity of inhaled pollutant compounds.

APPROACH: Mice will be exposed for periods of up to one year to carcinogenic metabolites such as benz(alpha)pyrene, alone or after adsorption to carbon particulate. Endpoints will include lung and other tumors, chronic lung disease, behavior and neurophysiology.

14. FURTHER INVESTIGATIONS WITH A 2-STAGE MODEL DESIGNED TO DETECT LOW POTENCY BLADDER CARCINOGENS AND CO-CARCINOGENS

Hicks, R. M., Severs, N., Chowaniec, J., University of London, The Middlesex Hospital Medical School, Dept. of Cell Pathology, *Cleveland St., London W1P 6DB, England, United Kingdom*

OBJECTIVE: To further develop our rabbit bladder model for detecting weak bladder carcinogens and co-carcinogens and to investigate the biological prinicples underlying multistage carcinogenesis, initiation and promotion.

APPROACH: We have developed a rat model which employs the principle of 2-stage carcinogenesis, to test compounds with suspected carcinogenic or co-carcinogenic potential for the bladder. The urothelium is exposed to a low dose of a known bladder carcinogen, and the test compound then administered to the animal by an appropriate route. If the test compound is also organotropic for the bladder and is either a solitary carcinogen, co-carcinogen or a promoter, it will increase the yield of tumors obtained with the low dose of the initiating carcinogen on its own. The model is also being used to investigate the dose response of the urothelium to a) the initiator and b) some of the test compounds; to determine the duration of the initiating effect; to compare the effect of single and 'split' doses of the same carcinogen; to identify useful and reliable initiators of carcinogenesis in the urothelium; and to study in depth the subcellular changes associated with the early, clinically 'silent' stages of neoplastic growth in the urothelium.

PROGRESS: N-methyl-N-nitrosoures (MNU) was used as the initiating carcinogen for a series of experiments. Saccharin and cyclamate both greatly increase the yield of bladder cancers in the MNU-initiated bladder and we conclude these compounds are weak initiators but powerful promoters of carcinogenesis in the rat bladder. 2-naphthylamine was both synergistic with

MNU and also a solitary carcinogen producing an incidence of approximately 25 percent bladder cancer when tested at high doses on its own. Coffee and caffeine did not significantly increase the tumor yield after initiation with MNU.

15. STUDIES ON EXPERIMENTAL BLADDER TUMORS

Cohen, S. M., Friedell, G. H., Jacobs, J. B., Murasaki, G., Lilja, H. S., St. Vincent Hospital, Dept. of Pathology, *25 Winthrop St., Worcester, Massachusetts*, 01604, United States of America

A reliable, highly reproducible model of bladder cancer in Fischer rats using the carcinogen N-(4-(5-nitro-2-furyl)-2-thiazolyl) formamide (FANFT) was developed. The natural history and the reversibility or irreversibility of early lesions were determined. It was also demonstrated that the carcinogenic process could be divided into 2-stages with properties similar to the classical murine skin carcinogenesis model of initiation and promotion. FANFT fed for 6 weeks was used as initiator and sodium saccharin and DL-tryptophan demonstrated promoting properties in this model. We propose to evaluate this model with regard to other criteria for initiation and promotion in urinary bladder carcinogenesis including irreversibility of initiation, reversal of order of administration of initiator and promoter, and length of time that promotion administration is required. The possible co-carcinogenicity of sodium saccharin and L-tryptophan will be evaluated. Mechanisms involved in initiation and promotion will be evaluated. Induction of bladder epithelial proliferation appears to be prequisite for initiation. Moreover, increasing the level of cell proliferation enhances the effectiveness of a given dose of initiator. Attempts will be made using increased proliferation to develop a model requiring a single dose of orally administered initiator. The anti-promoting activity of 13-cis-retinoic acid will be evaluated. A marker of abnormal cell proliferation in this model is the appearance of pleomorphic microvilli on cell surfaces as detected by scanning electron microscopy. Development of a short term assay for evaluating chemicals for activity toward the bladder will be attempted using pleomorphic microvilli as a marker. Identification of other markers, particularly histochemical assays for use in evaluating the carcinogenic and differentiation process in the bladder will be attempted.

16. THE EFFECT OF METHYLMERCURY ON TRANSPLACENTAL TUMOR INDUCTION WITH NITRITE AND ETHYLUREA

Nixon, J. E., Oregon State Higher Education System, Oregon State University, Environmental Health Sciences Center, Dept. of Food Science & Technology, *126 Agriculture Hall, Corvallis, Oregon*, 97331, United States of America

This project has three objectives: 1) to establish that methylmercury (MeHg) amplifies the toxicity and carcinogenicity of the model rat transplacental system, and that the effect is dose dependent; 2) to ascertain the optimum conditions for the model in terms of time of exposure, and the relationship between dose of N-nitroso compound and dose of MeHg for maximum MeHg effect; and 3) to determine the specificity of the MeHg effect to the transplacental system, to other N-nitroso compounds, and to other species.

Experiments will involve oral administration of sodium nitrite (NO2) and ethylurea (EU) or other amines, precursors of ethylnitrourea and other N-nitroso compounds, to pregnant rats exposed to dietary MeHg. Effects on reproduction and survival of progeny and on type, incidence, and latent period of neoplasms produced in the progeny will be determined. Preliminary experiments with progeny derived from dams fed NO2 plus EU and 10 ppm MeHg prior to parturition demonstrated that litter rate and survival were dramatically reduced, and the latency period for induction of neural tumors was decreased compared to progeny from control dams fed only NO2 plus EU, or MeHg. The data derived from this project will provide insight into factors that effect the toxic synergism of multiple dietary toxicants.

17. CARCINOGENESIS BY ISONIAZID AND HYDRAZINE ASSOCIATED WITH SODIUM NITRITE

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OBJECTIVE: To investigate the carcinogenic action of isoniazid and hydrazine sulphate associated with sodium nitrite in BALB/c mice.

APPROACH: Intact virgin, adult and newborn, BALB/c mice of both sexes were treated with isoniazid and hydrazine sulphate combined with sodium nitrite.

The pure non-sterile chemicals were administered by stomach tube, at the maximum tolerable dose. The treatment was started at 8 weeks in adults and at 24 hours in newborns. Mice were allowed to survive until death or killed when moribund. Lung, liver, endocrine glands and any organs suspected of tumor were removed at autopsy.

The results were reported at the VI International Conference on Cancer, Perugia University, 1977.

PROGRESS: Sodium nitrite alone was not carcinogenic, but when administered with hydrazine sulphate or with isoniazid, it increased the malignancy of pulmonary tumors and acted also on other organs such as cardiac stomach, liver and lymph nodes, particularly in newborn BALB/c mice.

The hydrazine sulfate-sodium nitrite reaction is being investigated.

RESULTS: A report on the 'Carcinogenesis of Isoniazid and Hydrazine in Association with Sodium Nitrite' was presented to the Sixth Quadrennial International Conference on Cancer 'Tumours of Early Life in Man and Animals' - and this has just been published.

18. IN VIVO BLADDER CARCINOGENESIS OF NITROSOAMINES

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The immediate objective of the proposed investigation is to ascertain whether urine contains a factor(s) which serves as a promoting agent(s) in urinary bladder neoplasia. Our studies are based on the hypothesis that urine may contain minute amounts of carcinogen(s) derived from either exogenous or endogenous sources and that these induce cancer only when they are present in sufficiently high concentration or act in concert with a second substance in urine which may serve as a co-carcinogen or as a promoter.

Available evidence indicates that the urine contains a number of substances which are known to stimulate cell growth, and it is conceivable that some of them may play the role of either tumor promoter or co-carcinogen. The proposed investigation is intended 1) to study the role of urine, specifically its possible role as a co-carcinogen and/or a promoter - as a study model, heterotopically transplanted bladders (HTB) of rats diverted from urine flow will be used; 2) to study the role of urinary constituents as possible co-carcinogens and/or promoters - the substances to be tested will be epidermal growth factor and polyamines; and 3) to test suspected proximate carcinogens using HTBs as a model.

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19. TUMOR PROMOTER AND CHEMICAL CARCINOGENESIS

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OBJECTIVE: Screening of tumor promoters from environment.

METHODS: a) ODC-induction in mouse skin; b) cell adhesion of HL-60 cell; c) differentiation of Friend erythroleukemia cell.

PROGRESS: Teleocidine B from Streptomyces was believed to be one of the naturally occurring tumor promoters.

20. CELL CULTURE STUDIES OF ENVIRONMENTAL PROMOTERS

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Agents, termed promoters, have been identified which, though noncarcinogenic per se, are able, either on mouse skin or systemically, to induce tumorigenesis specifically when administered subsequent to subcarcinogenic doses of chemicals and UV radiation. Furthermore, promoters show this activity even when administered long after carcinogen treatment, at times when DNA repair activity has been suggested to have terminated. Such observations have led to the proposal of an ubiquitous two-stage model of carcinogenesis by chemicals. At present few promoters of environmental relevance have been identified, even though their significance is probably considerable, since they may render tumorigenic doses of carcinogens regarded per se as offering little cancer risk, and whose removal from the environment would be impracticable on physical or economic grounds. The ability of promoters to enhance carcinogen-induced mutation frequencies in cultured V79 cells, discovered in our laboratory, suggests that the latter system has considerable potential in detecting environmental agents with promoting activity. Parallel characteristics of promoter action in animals and in V79 cell culture further suggest that the latter will be valuable for examining the mechanism of action of promoters.

The study will consist of three phases: a) Study of various parameters, including exogenous metabolic activating systems, determining mutation frequencies in the V79 cell mutagenesis system. b) Determination of the effect of tumor-promoting agents on carcinogen-induced mutation frequencies. c) Determination of the effect of promoters and nonpromoting analogs on biochemical parameters, implicated in tumor promotion, in V79 cells.

21. QUANTITATIVE STUDIES ON CONCURRENT EXPOSURES TO NUMEROUS CARCINOGENS

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Experimental studies are conducted on concurrent exposure of appropriate biological targets to numerous carcinogens of different classes, each administered at doses which would be expected to produce low or undetectable effects in the chosen system. Quantitative exposure/response studies are addressed to test the hypothesis that many different carcinogens can act synergistically to induce a significant level of carcinogenic response when given concurrently at individually subeffective doses. Mechanisms of action are studied to determine the most effective conditions of synergism. The biological models selected for these studies include in vitro assays for mutagenesis and neoplastic cell transformation and whole animal models. Analysis of dose-response relationships in carcinogenesis is conducted on data collected in a comprehensive literature survey, and on dose-response results obtained experimentally in the selected biological systems.

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B. COCARCINOGENIC EFFECTS OF IRRADIATION AND CHEMICAL CARCINOGENS

22. ENHANCED ORAL CARCINOGENESIS BY LOW DOSE X-RADIATION

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The tissues of the human oral cavity are frequently exposed to repeated low doses of known and suspected carcinogens such as tobacco and x-radiation from diagnostic medical and dental sources. Prior studies in our laboratory have shown synergy between concurrent applications of DMBA and exposures to modest levels of x-radiation in hamster cheek pouch tumorigenesis. This synergy was manifested by greater tumor incidence, shorter latent period, and greater tumor volume in animals receiving combined treatments than in animals receiving either treatment alone. We have also shown that low-level x-radiation causes a significant late increase in pouch vascular volume and that there is a significant interaction between the effects of x-radiation and DMBA-induced tumors on pouch functional vasculature. Ongoing studies are examining the effects of decreasing radiation doses on DMBA-induced tumor incidence and progression as well as the possible mechanistic role of radiation-induced hemodynamic changes in the induction and progression of these tumors. Young adult male Syrian golden hamsters have received various doses of DMBA to the cheek pouch accompanied by repeated exposures of 1-20R x-radiation at weekly intervals. The temporal relationships between applications of the chemical and radiation have varied. Animals are being observed for varying lengths of time; tumor latency, volume, incidence, histopathology and hemodynamics are being studied using observational, histopathological and radioisotopic techniques. It is anticipated that the results of these studies will clarify dose-response relationships and time-dose relationships of low level x-radiation in this system, leading to a more quantitative basis for calculations of human radiation risk estimates. Additionally, the data should provide information on the nature of radiation induced hemodynamic changes in the induction of epithelial neoplasms by low level exposures to multiple environmental carcinogens.

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23. CARCINOGENIC INTERACTIONS

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Over the next several decades a variety of energy sources will be used for the production of power. With these varied energy sources, workers and the public will be exposed to many different types of carcinogens at any one time. Although many of these carcinogens are being studied separately, little work has been done on their potential interactions. Because of this, the potential risks which may result from the interactions of low dose and dose rate exposures of ionizing radiation and chemicals cannot be assessed from presently available data. The objective of this project is to examine the nature of the interactions between radiation and chemicals at low doses and dose rates with particular emphasis on the potential tumor promoting or cocarcinogenic activity of low dose rate radiation exposures. Specifically this study will examine the cocarcinogenic or tumor promoting effects of low dose rate irradiation on the development of mammary adenocarcinomas in BALB/c mice after an initial exposure to low doses of 7,12-dimethylbenz(a)anthracene. These studies will allow the establishment of general principles which can be applicable for the assessment of risks for interactions in humans.

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24. CARCINOGENIC INTERACTIONS OF RADIATION AND CHEMICALS

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The objective of this project is to examine the nature of the interactions between radiation and chemicals at low doses and dose rates with particular emphasis on the potential tumor enhancing or cocarcinogenic activity of low dose rate radiation exposures. Specifically this research will examine: 1) whether the carcinogenic effectiveness of the radiation dose influences of a chemical carcinogen and low dose rate radiation; 3) the influence of radiation quality on these interactions. In these studies the effects of varying doses and dose rates of 137Cs gamma ray

of fission neutron irradiation on the development of mammary adenocarcinomas in BALB/c mice after an initial exposure to low doses of 1.12-dimethylbenz(a)anthracene will be determined.

25. MODIFICATION OF CHEMICALLY INDUCED CANCERS IN ANIMALS WITH LONG-TERM EXPOSURE TO LOW LEVEL MICROWAVE FIELDS (ABBREV)

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Rapidly growing environmental and occupational exposure to microwave radiation calls attention to the possible specific (non-thermal) biologic effect of this radiation, including delayed genetic phenomena and the possibility of neoplastic transformation of irradiated cells. Influence of long-term low-level microwave irradiation on cell mediated immune reactions is also postulated. These effects may differ, depending upon frequency of microwaves, time of exposure and power densities used. The following subjects will be elaborated in the project: 1. Development of skin cancer induced by 3,4-benzopyrene in mice exposed for 1-4 months to non-thermal (5 or 15 MW/cm2) power densities of 2450 MHz microwaves (continuous and pulse modulated waves); irradiation with microwaves will be performed prior to or simultaneously with treatment with 3,4-benzopyrene; cancer development will be evaluated macroscopically (7-range scale) and microscopically (routine histology and histochemistry). 2. Development of primary hepatomas in mice injected intraperitoneally with di-ethyl-nitroso-amine /DENA/ and exposed to 2450 MHz microwave field (as above). 3. Natural antineoplastic resistance (cancer lung colonies, natural killer activity) in mice exposed to low level microwave fields.

26. INTERACTION OF ULTRAVIOLET LIGHT AND CHEMICAL CARCINOGENESIS

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OBJECTIVE: Determine the effect of the interaction of UV irradiation and other environmental carcinogens on DNA conformation and DNA repair mechanisms.

APPROACH: Treat mammalian cells in culture with various polycyclic hydrocarbon carcinogens and then with UV irradiation. Determine effect on DNA repair response, DNA conformation, and stability of DNA and DNA-protein complexes.

27. RADIATION CARCINOGENESIS

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Our research with asbestos uses both Canadian and Rhodesian chrysotile fibers injected intratracheally, intrapleurally, or interaperitoneally, and uses the induction of mesothelioma or lung cancer as an endpoint. In one series, both methylcholanthrene and asbestos fibers have been injected to determine whether or not these diversely-acting carcinogens are synergistic. Both rats and mice are used. Some animals will be killed early to study the early precancerous cellular reaction. Glass wool fibers are used as control material since these are not known to induce mesothelioma. Few cancers develop in less than 300 days. In some animals, tumors have not appeared after 700 days. Our objectives are to determine the histologic changes involved in the development of mesothelioma, to determine whether synergism exists between the chemical carcinogen methylcholanthrene and asbestos, whether there is synergism between ionizing radiation and asbestos. We hope to determine how the pathologic sequences vary in the reactions to asbestos (which may culminate in mesothelioma) and those to glass wool (which do not). All of the rats treated with combined asbestos and methylcholanthrene in our studies of carcinogenicity have died. Histologic sections have been prepared and studied. Macrophages are the chief cellular response to asbestos, although some fibrosis occurs. Giant cells are formed more rarely in relation to asbestos in rats than in man. Compilation of the data from this group is in progress. Studies of asbestos-containing tissues by phase microscopy or polarized light show little advantage over those made by standard light microscopy, but electron microscopy is helpful. The protocols for injection of Canadian and Rhodesian asbestos and glass fibers in mice have been carried out, and most of the mice are still alive although a few of them have died and have been studied.

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28. COMBINED CARCINOGENIC EFFECT OF RADIATION AND CHEMICALS

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OBJECTIVE: To clarify the mechanism of additive carcinogenic effect of radiation and chemicals.

APPROACH: Target is the mouse skin. The skin was irradiated with low-penetrating beta rays in combination with painting with a chemical (e.g., 4-nitroquinoline 1-oxide, 4NQO). Skin tumor formation was then observed.

PROGRESS: With beta rays alone, the effect of dose-fractionation was found. The optimum condition for skin carcinogenesis is a repeated irradiation with 500-1000 rad. Single irradiation with 3000 rad alone did not produce a tumor. However, the latent effect persisted over one year and could be induced by combination with painting with 4NQO of sub-carcinogenic dose.

29. RADIATION AND CHEMICAL IN VITRO MALIGNANT TRANSFORMATION

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The basic objective of this research program is to gain specific information about the mechanisms of radiation carcinogenesis using in vitro assay system. Previous results indicate that X-ray transformation in vitro can be thought of as involving at least two steps: 1) the fixation of the transformed state (presumably irreversible DNA damage) and 2) its subsequent expression in terms of morphologically altered cells. We will investigate in detail the conditions necessary for fixation and ultimate expression of the cellular damage induced by ultraviolet light and ionizing radiation which results in malignant transformation of cells. In particular, we plan to study the role that DNA repair processes play in radiation-induced transformation and the interactions between radiation and chemical agents in malignant transformation. At present, little information is available on the interactions between radiation and other agents from in vivo carcinogenesis studies or in vitro transformation work, although human populations are continually being exposed to a variety of carcinogenic agents as well as to other agents that can influence carcinogenesis.

The cell system we will use is an established mouse embryo cell line which has been used for several years in this laboratory for radiation transformation experiments and is particularly appropriate for studying interactions between radiation and chemical agents. The specific chemical agents we wish to study have known interactive effects on carcinogenesis in vivo; studies in an in vitro system in which cell proliferation and other environmental conditions can be precisely controlled should yield further information about the mechanisms of carcinogenesis.

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30. RADIATION AND CHEMICAL CARCINOGENESIS

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OBJECTIVE: To understand the chemical effects of radiation on important cell components, and to probe the molecular mechanisms whereby radiation and/or chemical carcinogens produce cancer, using model chemical systems.

APPROACH: Radiation chemical damage to biological molecules, particularly DNA membrane phospholipids and their components, is produced by a 60Co gamma ray source, in the

presence and absence of chemical carcinogens. The radiation products are isolated and analyzed using HPLC, to determine the mechanism of radiation damage, the involvement of chemical carcinogens, and the extent of any combined or synergistic effects of radiation and chemical carcinogens. Kinetic studies using the technique of pulse radiolysis are being employed to measure the reactivity, selectivity and the nature and extent of involvement, of free radicals, particularly the hydroxyl radical, superoxide anion, singlet oxygen and other forms of activated oxygen, in cancer induction, transformation, or carcinogen activation, at the molecular level.

C. COCARCINOGENIC EFFECTS OF CHEMICAL CARCINOGENS AND VIRUSES

31. ANALYSIS OF MURINE LEUKEMIA VIRUS PRODUCTION IN THYMIC LYMPHOMAS INDUCED BY METHYLNITROSOUREA AND/OR X-IRRADIATION

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Methylnitrosourea (MNU) induces tumors in the thymus glands of C57BL mice whereas many other methylating agents do not. As the majority of naturally occurring or induced thymic lymphomas in mice are due to infectious murine leukemia virus (MuLV), including irradiation-induced thymomas (a system in which thymotropic MuLV is activated by X-rays), it is possible that the effects of MNU treatment could be a result of the activation of endogenous viral genomes. We are therefore measuring the production of MuLV in control, MNU, X-ray, and MNU plus X-ray treated mice to investigate whether virus production is a prerequisite to tumor production or whether it is a fortuitous side-effect of the drug treatment. To date, the results on thymoma extracts from small numbers of samples do not implicate virus in the MNU-treated mice. Seventy-five percent of the MNU-treated mice contained MuLV of xeno-tropic (or polytropic) host range only, compared to 17 percent in the MNU (low dose) plus X-ray group and 12 percent of X-ray treated mice; however, two of three mice in the control group also showed evidence of virus of this host range. On the other hand, viruses of ecotropic (including thymotropic) host range were more prevalent in mice exposed to X-ray (25 percent) and X-ray plus MNU (50 percent) than in those receiving MNU alone (0 percent). It is necessary to extend this investigation unitl statistically significant sample sizes have been tested in order to conclude whether or not activation of endogenous viruses plays a role in the etiology of MNU-induced thymomas.

32. POSSIBLE NEOPLASTIC EFFECTS OF NON-NEOPLASTIC VIRUSES

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Thymic lymphocytic lymphoma develops in mice spontaneously and in this case it is apparently induced by endogenous viruses (MuLV) or it can be induced by skin painting with 3-methylcholanthrene (MCA). The factors possibly responsible for the disease induction either by the virus or the carcinogen are being investigated.

Expression of endogenous MuLV: A high incidence of spontaneous lymphomas has been observed so far only in inbred strains (AKR and C58) which early in life already express a high level of endogenous MuLV. Consequently, strains which express low MuLV rarely develop the disease spontaneously but some (RF, DBA/2, DBA/1) respond to MCA painting with a high incidence of lymphomas. Whether this lymphomagenic effect might involve the carcinogen increasing the level of MuLV expression is being investigated by means of different techniques.

The induction in mice of the enzyme system aryl hydrocarbon hydroxylase (AHH) by MCA: Whether or not MCA exerts this effect is governed by the Ah locus, and this in turn influences the neoplastic effects of the carcinogen. Thus, whereas a high rate of spontaneous lymphoma occurs in AHH inducible (C58) as well as in AHH noninducible (AKR) strains, MCA induces the disease only in AHH-noninducible strains. However, some of these strains are highly resistant to this lymphomagenic effect. This indicates that the AHH non-inducible phenotype only contributes conditions favorable for the lymphomas to develop in the MCA-painted mice but that the actual occurrence of the disease is governed by genes unrelated to the Ah locus. Studies are in progress to identify these genes and to determine whether they might influence the spontaneous development of the lymphomas.

33. ENDOGENOUS VIRUS EXPRESSION AND LEUKEMOGENESIS

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In previous work I have demonstrated that RF mice inherit genes that code for two discrete effects on endogenous MuLV metabolism and leukemogenesis. One is a single gene, closely linked to the Fv-1 locus, that coordinately restricts xenotropic virus expression at 6 months of age and subsequent spontaneous leukemogenesis. This gene is dominant in the AKR x RF F1 hybrid mouse, and its restriction is reversed by treatment of both RF and AKR x RF F1 mice with 3-methylcholanthrene. This gene may be the Fv-1 gene itself. The second genetic effect results in low ecotropic virus titers and complete absence of xenotropic virus expression in RF mice. These phenotypes are recessive in the AKR x RF F1 mouse, and are unaffected by 3-methylcholanthrene treatment. The aim of the experiments proposed in this application is to probe the mechanisms of action of these mouse genes. The techniques to be employed include genetic crosses, assays for endogenous MuLV expression in mice (the UV-XC plaque assay for ecotropic virus, and a fluorescent focus assay for xenotropic virus), subcutaneous thymus grafting, in vitro and in vivo infection of mouse cells with MuLV, and renaturation kinetics of radioactively labeled viral cDNA in the presence of excess leukemic cell DNA. The proposed studies will investigate the interrelationships of mouse cellular genes, endogenous type-C viruses, and chemical carcinogens in the process of leukemogenesis.

34. THE ROLE OF ENDOGENOUS RETROVIRUSES IN LEUKEMOGENESIS

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The objective is to investigate the biological and molecular biological nature of a highly leukemogenic endogenous murine retrovirus (DMBALV) recoverable from chemical carcinogen induced thymic lymphomas in strain CFW/D mice. The relationship of this virus to the only other endogenous virus detectable in CFW/D mice, a xenotropic virus, is also being investigated.

Nucleic acid sequence homology between DMBALV, the xenotropic virus, and Moloney MuLV is being investigated using standard molecular hybridization techniques including the use of viral gene specific cDNA probes. The number of copies of nucleotide sequences representative of each of the viruses in the DNA and RNA of viral, chemical and x-irradiation induced tumors is being determined. Mapping studies of the DMBALV and XeMuLV nucleotide sequences relative to one another in normal thymus and bone marrow cell DNA and tumor cell cDNA are also being carried out. The biological effects of DMBALV on thymus cell populations are also being studied. The approach has been to establish the viral induced tumors in culture and to show that some of the tumor cell lines are able to function as normal immunocompetent thymus cells.

The viral nature of DMBALV has been determined. It has the properties of a C-type virus (density of 1.16 gm/cc, 70 RNA genome which on denaturation gives rise to 35S subunits). The virus is unable to productively infect any cell line in culture and is only infectious in vivo when inoculated directly into the thymus. The latent period for tumor induction is short: tumors are detectable 30 days post injection. Hybridization studies indicate that the XeMuLV and DMBALV share at least 50% of their nucleotide sequences and both viruses share at least 60% of the sequences present in MoMuLV and MuSV. Ten DMBALV induced tumors have been established in culture and are characterized by the presence of a modal chromosome

number of 41, due to a specific lesion involving trisomy of chromosome 15. All are highly sensitive to anti-thymus cell serum. Some of the tumor cell lines have been shown to be immunocompetent in that they can be specifically activated upon contact with antigen in an analogous manner to normal, non-transformed thymus cells.

35. C-TYPE VIRUS ACTIVATION DURING CHEMICALLY INDUCED LEUKEMOGENESIS IN MICE

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OBJECTIVE: The objective of this study is to analyze in detail the activation of C-type inbred strains of mice during the development of chemically induced leukemias and to further clarify the role of the endogenous C-type virus in leukemogenesis.

APPROACH: Mice are subjected to a standard treatment with a chemical carcinogen which will induce a non-thymus derived leukemia with a relatively short latency period. Sets of treated and non-treated animals are sacrificed at short intervals during the development of leukemia. Concentrations of p30 in blood and spleens are determined by radioimmunoassay and isolation of virus is attempted from the splenocytes. The host range as well as leukemogenicity of the isolated virus is investigated and the protein patterns are determined in SDS polyacrylamide gel-electrophoresis.

PROGRESS: Several virus isolates have been obtained from both leukemic and non-leukemic mice. These are mainly ecotropic and N-tropic viruses. Differences have been observed in electrophoretic mobilities of proteins from viruses isolated from leukemic and non-leukemic animals; differences have also been observed with respect to the rate of virus spread.

It will be attempted to correlate these different parameters with the development of the leukemia, which is evaluated by transplantation of splenocytes to syngeneic, partially immunodepressed mice.

36. EVALUATE THE COINSULT EFFECTS OF LIGHT AND TUMOR PROMOTING AGENT ON INDUCTION OF ENDOGENOUS VIRAL GENES IN MICE

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Previous work has shown that induction of endogenous type C RNA virus occurs after exposure of mouse cells to ultraviolet (UV) radiation, certain chemicals, and biological agents, such as UV-irradiated or photodynamically treated herpes simplex virus. Exposure of Al-2 cells, derived from the BALB/c mouse, induces endogenous xenotropic type C virus as determined by the sensitive infectious center assay on normal rat kidney cells. Moreover, the effects of caffeine, macromolecular synthesis inhibitors, and protease inhibitors have been utilized to elucidate the mechanism(s) of cellular control of integrated viral gene expression. Since interactions between light radiation and chemicals may be toxic and potentially carcinogenic, bioeffects information is needed to adequately evaluate such interactions. The research proposed in this project will examine the effect of light and tumor promoters on induction of endogenous viral genes in mouse processes and since carcinogenesis involves alteration of normal cellular regulation, the viral induction studies should yield information important for understanding light- and light-chemical-induced carcinogenesis.

37. IN VITRO CHEMICAL CARCINOGENESIS AND CO-CARCINOGENESIS IN HUMAN CELLS INITIATED BY HYDRAZINE AND POLYNUCLEAR COMPONENTS OF JET FUEL

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AF function - the AF uses many unique chemicals to obtain optimum performance of its aircraft and missile weapons systems. National legislative and regulatory controls on the use of energetic chemicals have necessitated a step-function increase in the need to define safe exposure limits and handling practices for these chemicals concurrent with the acquisition of the weapon system. Deficiency - there is a need to investigate mechanisms and to devise methodologies to predict, detect, measure and modify pharmacological manifestations of the unique chemicals associated with USAF weapon systems in order to protect personnel from health and performance degradation. Research is needed to determine and identify actual molecular mechanisms of chemical carcinogenesis. Objective - the objective of this research is to elucidate the mechanisms producing changes in cell chemistry, DNA and RNA and to correlate these with the metabolic and biochemical state of the cells following exposure to hydr[illegible]es and PNH's. How research contributes -this research co[illegible]outes directly to Subarea 1.8, 'Environmental Protection and Toxicological Hazards.'

Studies will examine the cytotoxic and carcinogenic potential of hydrazine and polynuclear hydrocarbons in synchronized and nonsynchronized normal human cells, investigate damage to cellular DNA by studying unscheduled repair kinetics and alterations in cellular-associated RNA-directed DNA polymerase activities, investigate whether the suspect carcinogens act synergistically with viruses to enhance virus transformation of human cells, investigate whether these chemicals activate endogenous RNA-C-type virus expression in human cells, determine whether these chemicals damage cellular DNA, providing additional sites for virus integration. New approaches will include autoradiographic measurement of chemical uptake and cytoplasmic binding using electron microscopy, and determination of the effect of hormones on viral molecular probes.

38. EFFECTS OF CHEMICAL CARCINOGENS ON HEMOPOIESIS, IMMUNOPOIESIS, AND VIRAL ONCOGENESIS

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The objective is to study the interactions of leukemia causing viruses with radiation and/or chemical carcinogens. The studies will be carried out in vivo, taking into consideration effects on the kinetics of the virus target cells of the animal and effects on the immune status of the animal. Effects on cell kinetics will be measured in the whole tissue using standard techniques involving tritiated thymidine labelling and measured in the pluripotent stem cells using CFU-S assay techniques. Immune activity will be evaluated using techniques which measure both humoral and cellular immune function. Assay of the hematopoietic tissue for viral infection, transformation and production will be carried out using the X-C syncytia formation technique.

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39. MECHANISMS OF TUMOR PRODUCTION GROWTH FACTORS AS MEDIATOR OF TUMOR PROMOTION

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This project aims to isolate and characterize putative endogenous tumor-promoters and to develop a rapid and economical assay for identification of exogenous and endogenous tumor-promoters. The following activities were specifically pursued: (1) To elucidate the mechanism of TPA induced inhibition of EGF binding (TIEB). To search for compound(s) which can reverse and modulate TIEB at non-toxic doses. (2) To isolate and characterize membrane receptors of phorbol esters. (3) To search for endogenous ligand(s) for phorbol receptors, isolate and characterize them. (4) To isolate and characterize growth factor(s) induced by biologically active phobol esters. (5) To test whether EGF and other growth factors enhance the carcinogenesis in-vivo and in-vitro. (6) To investigate whether TPA induces or enhances the expression of endogenous oncogenic cellular information. (7) To study whether retroviruses act as promoters in the carcinogenesis started by chemical or physical agents. (8) The reversal of anchorage independent growth of transformed cells by differentiation inducing agents.

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brane receptors. Nature 279:387-391, 1979. Keski-Oja, J., Shoyab, M., De Larco, J. E., and Todaro, G. J. Rapid release of fibronectin from human lung fibroblasts by biologically active phorbol esters. Int. J. Cancer 24:218-224, 1979.

40. IN VITRO STUDY OF THE NATURE OF INTERACTION BETWEEN CHEMICAL AND VIRAL CARCINOGENS

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OBJECTIVE: Develop a human tissue culture system that will respond quantitatively to chemical carcinogen treatment, to feline sarcoma virus (FSV) directed focus formation, to chemical carcinogen FSV virus interaction and to steroid-carcinogen-virus interaction.

APPROACH: All chemicals steroids and viruses will be suspended in media and added to target cells at predetermined dose levels. For chemical-virus experiments, cells are infected with a dilution of virus which produces a known number of focus-forming units. The effect of the carcinogen is shown by the deviation in foci number from expected control values. A test for malignancy of cells isolated from transformed foci will be carried out by injecting these cells I.D. into mice.

41. TRANSFORMATION OF ORAL MUCOSA BY HERPES SIMPLEX VIRUS

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The current research is intended to examine the ability of herpes simplex viruses, alone and in combination with chemical carcinogens, to function in transformation of the oral mucosa. The studies concentrate on changes in the cell surface structures and components, particularly alterations in lipids, glycolipids and glycoproteins, that occur in the early time periods following infection. These components are known to affect cell social behavior and tissue differentiation, and change following viral and chemical induced transformation. Mucosal tissues are grown in vitro and infected with herpes simplex viruses which have been shown to have the ability to transform cells. At varying periods of time following infection, the explanted tissues are harvested and examined for chemical changes in the cell lipid, glycolipid and glycoprotein componets. Neutral lipids and phospholipids are separated and identified by column, paper and gas chromatography and autoradiography. Glycolipids are to be separated by thin layer chromatography and autoradiography. Glycoprotein determinations are made by radioactively labeling glycoproteins which will subsequently be separated by acrylamide gel electrophoresis and radioactivity will be determined. Enhancement of transformation by chemical will be determined using cultures of epithelial cells infected with virus or treated with virus and a chemical carcinogen. The relative ability of the chemical to increase virus induced transformation will be determined. In addition, it is proposed to attempt to develop a method of culturing epithelial cells using conditioned medium or cell extracts, rather than the feeder layers of irradiated 3T3 fibroblasts. Morphological studies on epithelial tissues will be conducted at an ultrastructural level to supplement the analytic studies and demonstrate decreased numbers and altered shapes of microvilli and fewer as well as fewer and smaller intercellular junctions.

42. HERPES VIRUS INFECTION AS CO-FACTOR IN CARCINOGENESIS - CELL GROWTH STIMULATING FACTOR (ABBREV)

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OBJECTIVE: Previous research has shown that the growth of Yoshida sarcoma in rats and a syngeneic MCA-induced spindle cell sarcoma in mice is enhanced by a simultaneous HSV-2 infection of the animals. The cause of this phenomenon is currently being investigated.

APPROACH: It was found that monolayers of certain cell lines (mainly CV1 cells) synthesize a mitogen (DNASF) following their lytic infection with either HSV-1 or HSV-2 which is shed into the supernatant medium (SupV positive). This is demonstrated by an increased (3H)TdR incorporation of indicator cells growing in SupV positive as compared to those growing in supernatants of control cultures (SupV negative). Cell counts confirmed increased cell multiplication.

PROGRESS: DNASF is genetically non-specific and its production reaches a peak when infection of the cells is complete. It is not accumulated inside the cells.

Further investigations involve the purification and the characterization of the DNASF molecule and its activity in vivo. Furthermore, it is intended to clarify whether the production of DNASF is limited to HSV and whether all strains of HSV are capable of producing the factor.

43. IN VIVO COCARCINOGENESIS OF CHEMICALS AND VIRUSES

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The long term objective of this proposal is to examine carcinogenic perturbation of the transforming ability of viruses. Particular attention is being paid to the environmental carcinogens, the N-nitroso compounds. We are exploiting cocarcinogenic in vivo interactions and we are also measuring the effect of carcinogen on certain in vitro parameters of transformation. These latter experiments can be used to predict the in vivo behavior of transformed cell populations.

The current studies have devoted a large effort to the characterization of the enzyme plasminogen activator (PA) as a parameter of oncogenic transformation for cells transformed by herpes simplex virus type II (HSV-2). A strong positive correlation has been established to show that increased PA activity is predictive for the increased ability of a transformed cell population to form a tumor after subcutaneous injection of newborn hamsters. More importantly, for HSV-transformed cells, increased levels of this enzyme indicate that the resulting primary tumor will metastasize to lung tissue more rapidly and more extensively. Experiments are now in progress to use PA expression as a predictive enzyme to determine the effect of N-nitroso compound exposure on HSV-2 transformed cells.

BIBLIOGRAPHIC REFERENCES: Adelman, S.F., Howett, M.K. and Rapp, F. Quantification of plasminogen activator activity associated with herpesviruses-transformed cells. J. Gen. Virol., 50: 101-110, 1980.

44. INDUCTION OF MTV EXPRESSION UPON HORMONAL STIMULATION AND BY NON-VIRAL CARCINOGENIC TREATMENT OF BALB/C FEMALES

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OBJECTIVE: To measure the effect on MTV expression by treating mice with mammary tumor inducing agents such as X-irradiation, hormones (continuous exposure to prolactin) and urethane.

APPROACH: The expression of MTV-RNA is measured by molecular hybridization between MTV3H-cDNA and cellular RNA. The MTV proteins are measured by using sensitive radioimmunoassays for the MTV proteins gp52 and p27.

PROGRESS: Continuous exposure of female BALB/c mice to prolactin resulted in the induction of mammary tumors (68%) with 70 times more MTV-RNA than was present in spontaneously occurring mammary tumors in BALB/c mice. The latter contained 0.04 MTV-RNA genome equivalent per tumor cell. Treatment of BALB/c females with X-irradiation (200 rads WBR) or with urethane (0.1% in drinking water) resulted in mammary tumors with elevated MTV-RNA levels, but still less than one MTV-genome equivalent per tumor cell was detected. The combined treatment of X-irradiation and urethane resulted in the development of mammary tumors containing a 1724-fold increase of MTV-RNA. These mammary tumors contained, however, very low levels of MTV proteins, suggesting some kind of impairment at the level of translation.

45. MMTV IN SPONTANEOUS AND CARCINOGEN-INDUCED TUMORS

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Mammary tumorigenesis in mice is influenced by chemical carcinogens, hormones and the genetic complement. Among the factors in the genetic complement that appear to influence mam-

mary tumorigenesis is the endogenous, germinally-transmitted murine mammary tumor virus (MMTV). This proposal will examine the relationship between germinally-transmitted MMTV DNA and mammary tumorigenesis in several C3Hf sublines and C3H/StWi mice. Mammary tumorigenesis in C3Hf mice is strongly influenced by hormones. Several C3Hf sublines will be examined because C3H/Bi and C3H/St mice have different MMTV DNA sequences and because C3Hf/He and C3Hf/Ki tumors differ in their immunologic properties. Mammary tumorigenesis in C3H/StWi mice is not influenced by hormones but occurs following treatment with urethane or 7,12-dimethylbenz(a)anthracene. To examine the role of germinally-transmitted MMTV in mammary tumorigenesis in these mice, MMTV RNA expression will be quantitated in normal and neoplastic mammary tissues by molecular hybridization. The complexity of MMTV RNA will be examined by separation of MMTV RNA species on the basis of size and detection of MMTV RNAs by filter hybridization. The MMTV DNA sequences will be examined by restriction endonuclease mapping and correlated to MMTV expression. Recent studies have suggested that expression of specific genes is related to hypomethylation of the gene sequence. The relationship of methylation of MMTV DNA to MMTV RNA expression will be examined by digestion with isoschizomeric restriction endonucleases that differ in their ability to cleave methylated sequences. Through these studies a better understanding of the relationship of the germinally-transmitted MMTV to mammary tumorigenesis by hormones and chemical carcinogens will be obtained.

46. INFLUENCE OF CARCINOGENS ON VIRAL GENE EXPRESSION

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OBJECTIVES: To determine whether the non-viral carcinogens that do induce mammary tumors in mice do so by modification of MuMTV gene expression, or whether they act by an MuMTV independent, alternative pathway. In order to answer this question, we intend to study biochemical, virological and immunological parameters reflecting MuMTV gene expression at short intervals in individual mice, which are part of large groups, each of which is subjected to a different oncogenic stimulus, or to a combination of such stimuli.

47. A POSSIBLE VIRAL ETIOLOGY OF RAT MAMMARY TUMORS - EFFECTS OF IRRADIATION, HORMONAL STIMULATION AND URETHANE TREATMENT

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OBJECTIVE: To study the induction of mammary tumors in rats by roentgen irradiation or chemical carcinogens and to establish the presence of a rat mammary tumor virus.

APPROACH: Rats of four different strains, Sprague-Dawley, Long-Evans, R and U are roentgen irradiated, treated with urethane or stimulated by hormones by implantation of a pituitary gland under the kidney capsule. The animals are checked for the occurrence of mammary tumors. Tissue cultures of mammary tumors are tested for the presence of virus particles by electron microscopy, for viral reverse transcriptase and for mammary tumor inducing capacity in rats or mice. Milk and mammary tumors of rats are tested for antigens that cross-react with mouse MTV.

PROGRESS: In tissue culture medium of a mammary carcinoma of a U rat, virus-like particles were found that had a Mg ion-dependent reverse transcriptase activity when the artificial templates dGrC or dTrA were used. U rats injected with these particles did not show an increased tumor percentage, but injected BALB/c mice had 85% of mammary tumors at 18 months. Immunological cross-reactions of these particles with other retraviruses have not been observed yet. In some mammary tumors and in milk of rats of the U and R strains, antigens were found that cross-reacted with mouse MTV, both in immunodiffusion tests and in a radioimmunoassay for MTV p27.

48. ANALYSIS OF THE SHOPE PAPILLOMA-CARCINOMA SYSTEM

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The Shope papilloma-carcinoma system has great potential for the definition of basic biochemical phenomena which are critical to the neoplastic process. This is evident since in domestic and cottontail rabbits there is a virally induced progression from normal skin to papilloma to carcinoma. We have shown that multiple copies of the viral genome are conserved in all neoplasms and that some 5% of viral genetic information is transcribed in tumors of domestic rabbits (which characteristically do not produce virus). That multiple copies of the virus genome are present in viral superinfected chemically induced papillomas and in serially transplantable VX-2 and VX-7 carcinomas has also been shown.

In the present application we propose to determine: 1) Whether the same transcripts are present in all tumors. 2) Whether or not the viral DNA is covalently integrated into the host cell genome. 3) If there is a translation product in these tumors and if so, its basic properties (since it is likely to be involved in an induction and maintenance of the neoplastic state). 4) The viral specific macromolecular events which accompany malignant change induced by the virus in superinfected papillomas established by a chemical carcinogen. In addition, in related studies the effect of a tumor promotor on the expression of viral genes will be assessed. 5) If VX-2 and VX-7 carcinomas resemble the primary tumors with respect to viral gene expression. Should this prove to be the case, cultures of these cells will be intensively studied to determine the nature of the virus cellular relationship as it relates to these neoplastic cells. 6) If using additional methods, cell lines from the virus tumors can be established in vitro. Molecular hybridization, restriction endonuclease digestion, immunoprecipitation of radioactive materials and cell culture methods appropriate to epidermal cells will be the primary tools employed in these studies.

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49. CARCINOGENS AND PAPILLOMA VIRUSES IN BOVINE CANCER

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OBJECTIVES: Characterize, compare, and contrast the three types of papilloma virus isolated from cattle, viz: bovine cutaneous papilloma virus (BCPV), the classical bovine papovavirus; bovine alimentary papilloma virus (BAPV); and bovine udder papilloma virus (BUPV). Investigate alimentary squamous carcinomas found on bracken infested farms for the presence of papilloma virus genome. Make a similar study of the adenomas and adenocarcinomas found in the intestine. Set up experimental models in which the bracken fern component is replaced in 'carcinogen plus virus' type experiments by such compounds as azothioprene to try to gain some insight into the nature of the mechanism of interplay of the dietary factor and the virus.

50. INTERACTION OF CHEMICAL CARCINOGENS AND TUMOR VIRUSES IN ONCOGENIC TRANSFORMATION

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The objective of the present study is to characterize oncogenic transformation induced by chemical carcinogens and the interaction between chemical carcinogens and tumour viruses.

The objective is being approached both by in vivo and in vitro studies. Nude mice are used as animal models and as a source of cells for cell cultures. Mixed fuction monooxygenase activity of nude mice and cultured cells will be analyzed and compared to the susceptibility to chemical transformation. Interaction of chemical carcinogens and viruses will be analyzed by an in vitro transformation assay.

Preliminary analyses of the enzymes mentioned above have been performed (Laaksonen et al., 1979, to be published in Animal Quality and Models in Biomedical Research, Eds. A

Special and O. Muhlbock, Gustav Fischer Verlag, Stuttgart New York). Optimal conditions for transformation by 3-methylcholanthrene in nude mouse filbroblast cultures have been determined.

51. CARCINOGENESIS OF MAMMALIAN CELLS IN CULTURE

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The objective of this project is to determine the mechanisms of 'spontaneous' and carcinogen-induced malignant transformation in cultured cells. Emphasis is on the development of culture systems utilizing human epithelial cells to study the interaction of chemical carcinogens and viruses with cellular DNA, to define fundamental cytologic and other characteristics of carcinogenic change, and to provide quantifiable criteria of malignant conversion. The project this year consists of the following four studies: 1. Light-induced chromatid damage in relation to DNA repair and malignant transformation in culture. 2. Differential sensitivity of normal cells and their neoplastic derivatives to the lethal action of hydrogen peroxide. 3. Development of human epithelial cell systems for carcinogenesis studies. 4. Evaluation of in vivo assay techniques for determining the neoplastic state of cultured cells.

BIBLIOGRAPHIC REFERENCES: Sanford, K.K., Parshad, R., Handleman, S.L., Price, F.M., Gantt, R.R., and Evans, V.J.: Serum-induced chromosome damage and neoplastic transformation of mouse cells in vitro. In Vitro 15: 488-496, 1979. Price, F.M., Camalier, R.F., Gantt, R., Taylor, W.G., Smith, G.H., and Sanford, K.K.: A new culture medium for human skin epithelial cells. In Vitro 16: 147-158, 1980.

52. VIRAL INFECTION AND GENETICS IN SPONTANEOUS OR CHEMICAL NEOPLASMS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

II. INHIBITION OF CHEMICAL CARCINOGENESIS IN MODEL SYSTEMS

A. INHIBITION BY RETINOIDS AND PRECURSORS

53. SKIN CARCINOGENESIS AND RETINOIDS

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STATEMENT OF OBJECTIVE: To establish whether retinoids alter the development of skin carcinogenesis induced by chemicals and light.

DESCRIPTION OF WORK: Tumors will be induced in skin and oral mucosa by either DMBA or UV light exposure alone as well as in combination with cosmetics and other photosensitizers, employing established methods in control and experimental Syrian hamsters. Retinoids will be administered orally and intraperitoneally. Tumors and related biological samples will be obtained from Harvard Medical School, Boston. The following parameters will be determined: 1) Level of retinoic acid and its metabolites as well as endogenous retinol in epidermal tissues HPLC and spectrofluorometric methods. 2) Levels of aryl hydrocarbon hydroxylase in tissues of control and treated animals by the standard fluorescence method of Bevert et al. (J. Biol. Chem. 243, 6240, 1968). 3) Levels of free arachidonic acid, prostaglandins and cyclic AMP from control and treated animals by modified standard methods (GLC, radio-immunoassay). 4) Levels of cancer associated isoenzyme of serum galactosyl transferase in control and treated animals by polyacrylamide gel electrophoresis (Weiser et al., Proc. Natl. Acad. Sci. 73, 1319, 1976). In addition, this year (Fiscal 1980) special attempt will be made to determine the isomeric composition of retinoic acid isolated from tumor and control tissues. This work had not been performed before and should enable us to better understand the mechanism of action of retinoids in chemical carcinogenesis.

54. RESPIRATORY CARCINOGENESIS - MARKERS OF NEOPLASTIC DEVELOPMENT IN THE RESPIRATORY SYSTEM

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OBJECTIVES: 1. To define specific stages in the evolution of neoplasia in respiratory epithelium. 2. To develop and utilize in vivo-in vitro and in vitro model systems to define the quantitate cellular changes that identify particularly the preneoplastic stages of carcinogenesis. 3. To determine the effects of retinoids on the differentiation and progression of neoplasia in these systems, and thereby contribute to the understanding of the mechanism of action of vitamin A.

MAJOR FINDINGS: 1. Using the tracheal implant-organ culture-cell culture model, an experiment was initiated to study the growth behavior and progression to neoplasia in epithelial cell populations derived from specific lesions induced in tracheal implants by exposure to 200 microgram dimethylbenz(a)anthracene. To date, it has been determined that some cell populations can be identified by alteration in in vitro growth capacity and by differential response to vitamin A (no effect, stimulation, inhibition of growth in agar). 2. Experiments were initiated to further validate the tracheal organ culture-cell culture model as a system for quantitatively evaluating the appearance of morphologically altered foci (a preneoplastic marker) in epithelial cell populations derived from carcinogen-exposed tracheal explants. The effects of a single versus a split dose of benzo(a)pyrene on the number and time of appearance of these foci is under investigation. The incorporation of a growth selection step in the protocol has improved on the time to the identification of the morphologically altered foci. 3. Preliminary experiments have been carried out to determine the effects of benzo(a)pyrene on growing primary tracheal epithelial cell cultures. To date, it has been determined that: (a) Some cultures develop a greatly increased growth rate soon after carcinogen exposure. (b) Foci of tightly packed cells (morphologically-altered cells) are seen only in carcinogen-exposed cultures. (c) These foci can be identified under 'step-down' culture conditions within two weeks after a single exposure. (d) Only carcinogen-exposed cells are subculturable. It remains to be seen how long it will take to demonstrate neoplastic transformation in these cells, and how it will compare to the in vivo-in vitro and the organ culture systems described above. (Text Abridged.)

55. VINYL CHLORIDE CARCINOGENESIS

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OBJECTIVE: To study in vitro and in vivo carcinogenesis induced by vinyl chloride and vinyl derivatives and the action of retinoid derivatives on tumorigenesis.

APPROACH: Pluripotential fetal mouse liver cells will be treated in vitro with vinyl derivatives including vinyl chloride and vinyl bromide. Transformation will be evaluated by foci formation, growth in soft agar tumor production in vitro with the derivatives inducing unscheduled DNA synthesis used as a first screening test.

The effect of retinoid derivatives on the inhibition of tumorigenesis will be studied in vitro. Retinoic acid and its methyl ether will be assayed on cells after induction with vinyl derivatives and in prophylaxis.

The effect of retinoid and vinyl derivatives will be studied in vitro on the induction of ornithine decarboxylase which is presently one of the first transformation steps.

PROGRESS: We have developed a rapid and reliable assay for carcinogenesis using unscheduled DNA synnthesis by human amnion cells on solid supports. Styrene oxyde has been recognized as a potential carcinogen using this assay. Medical

surveillance of vinyl chloride workers indicates that this population is abnormal with respect to a very high percentage of elevated serum CEA values and other biochemical tests.

56. SUPPLY CHEMOPREVENTION RETINOIDS FOR EPITHELIAL CANCER

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Determine which retinoids are highly effective in inhibiting N-methyl-N-Nitrosourea (MNU) induced mammary carcinogenesis. Determine the time interval that may elapse either before or after the carcinogenic insult without impairing the efficacy of the retinoid as regards mammary carcinogenesis. Determine the efficacy of retinoids relative to hormone dependency of the tumors. The efficacy of the retinoid in inhibiting mammary carcinogenesis will be assessed by evaluating the following parameters of mammary carcinogenesis: percent incidence mammary cancer and benign mammary tumors; number of mammary cancers and benign tumors; number of invasive tumors; number of mitotic figures; and latent period of tumor appearance.

57. PREVENTION OF PANCREATIC CANCER IN ANIMALS BY RETINOIDS

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OBJECTIVE: To induce an incidence of carcinomas or preneoplastic epithelial lesions in pancreas of azerine-treated rats and N-nitrosobis(2-oxopropyl)amine-treated hamsters, and then to see if there is a decrease in the incidence, size, or number of such cancers or preneoplastic lesions in the rats and hamsters given retinoid-supplemented diets during the latent period of carcinogenesis.

58. INHIBITION OF ANTI-NEOPLASTIC CARCINOGENESIS BY RETINOIDS AND ANTIMUTAGENS

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OBJECTIVE: Several reports have shown that antineoplastic agents have a carcinogenic effect both in experimental animals and in man. Each antineoplastic agent causes tumors in its end organ system. This research program aims at analyzing the possible inhibition of antineoplastic agent carcinogenesis by retinoids and antimutagenic agents (quinacrine). Several reports have already shown that retinoids protect the epithelial cells from neoplastic transformation caused by several chemical carcinogens. Quinacrine is antimutagenic bacteria, murine and human cells in vitro.

APPROACH: Several groups of mice will be co-treated with cyclophosphamide, which is a potent antineoplastic agent, and retinoids or quinacrine in order to demonstrate the possible inhibition of tumor development.

59. CHEMOPREVENTION OF EPITHELIAL CANCER BY RETINOIDS

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The basic objective of these efforts is the evaluation of the efficacy of retinoids of differing chemical structures in preventing the development of epithelial cancer during its preneoplastic period. A number of target sites for such chemoprevention are anticipated: respiratory tract, urinary bladder, breast, and colon. Appropriate animal models are currently available in the literature for these organ sites. Carcinogens and retinoids necessary for these studies will be provided in most instances by the National Cancer Institute as available and in accordance with program priorities. Close coordination and mutual consultation by contractor and the NCI is expected in reaching the objectives of this contract.

60. CAROTENOIDS AS ANTI-TUMOR AGENTS FOR SKIN TUMORS

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Light, particularly the sunburn spectrum (UVB, 280-320 nm), has been implicated as an etiologic agent in the induction of skin tumors in animals and man. We have shown that the administration of carotenoid pigments to patients with erythropoietic protoporphyria can ameliorate the light sensitivity associated with this disease, and that the administration of these pigments to normal volunteers was found to have slight but statistically significant action in delaying the appearance of erythema caused by the sunburn range. We have also found that phytoene, a precursor pigment having absorption maxima in the sunburn range, significantly decreased the erythemal reaction in guinea pigs exposed to this radiation. In addition, we and others have shown that carotenoid pigments can inhibit the formation of free radicals which have been implicated in photocarcinogenesis. Therefore we have studied the effect of the systemic administration of several carotenoid pigments on the development of skin tumors induced by sunburn radiation and topical carcinogens in hairless mice.

We have found that the administration of beta-carotene, phytoene or canthaxanthin causes a delay in development, and inhibits significantly the development of tumors induced by UV-B (290-320 nm) per mouse, in hairless mice receiving these pigments. In addition, beta-carotene has an inhibitory effect on dimethyl benz (a)anthracene-induced tumors, but phytoene and canthaxanthin have no such effect. We now propose to extend our studies in two directions; 1) to investigate the possible mechanisms of action of carotenoid protection, by determining photo-product formation on DNA and cellular damage in animals receiving each of the pigments as compared to animals receiving placebo, and to study some aspects of the pharmacokinetics of these pigments, either alone or in combination, to establish the most effective administration methods for maximal tumor inhibitory effect. In addition, we plan to study the effect of crystalline preparations of the water soluble carotenoid, crocetin and other carotenoids as they become available, on the effect of UV as well as chemically-induced skin tumors.

B. INHIBITION BY ANTIOXIDANTS

61. MECHANISM OF ANTI-CARCINOGENIC EFFECT OF ANTIOXIDANTS

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The antioxidants such as t-Butylhydroxyanisol (BHA), t-butylhydroxy toluene (BHT), and ethoxyquin offer protection to mice from lung adenoma and other forms of cancer caused by the administration of benz(a)pyrene (BP) and dibenz(a,h)anthracene (DBA). However, the mechanism of this protection is not clear. The anti-carcinogenic effect of antioxidants could either be due to the activation of the enzyme systems (such as GSH-S-transferases) which are involved in the detoxification of highly electrophylic toxic metabolites of procarcinogens or the antioxidants are themselves metabolized by mixed function oxidases in the same manner as the carcinogens and the metabolites of antioxidants compete with 'ultimate' carcinogens for the binding with cellular macromolecules. The work proposed in this project is aimed at understanding the mechanism of the effect of anticarcinogenic action of antioxidants. The effect of oral administration of ethoxyquin, BHA and BHT will be studied on lung adenoma produced in mice by administration of BP and DBA. The effect of these compounds on the total GSH-S-Transferase of the lung and on the isozymic pattern of this enzyme will be studied. Besides kinetic characterization, immunochemical studies will be performed using antibodies raised against homogenous preparations of genetically distinct mouse lung GSH-S-transferases. Since in the mouse liver several genetically distinct isozymes are known, the effect of antioxidants on each one of the GSH-S-transferase isozymes of lung will be studied. GSH-S-transferase is known to bind chemical carcinogens, therefore, the binding of each of the mouse lung GSH-S-transferase and the metabolites of BA and BP will be studied. In addition, the effect of antioxidants on the mixed function oxidases which are responsible for the activation of chemical carcinogens and on the overall metabolism of GSH which provide in vivo substrate (GSH) for GSH-S-transferase

will be studied. Metabolism of antioxidants by mixed function oxidases will be studied by incubating the antioxidants with microsomal fraction and by characterizing the metabolites using modern physico-chemical techniques. Effect of these metabolites on the binding of carcinogen and GSH-S-transferase will also be studied.

62. PHARMACOKINETIC PARAMETERS IN CARCINOGENESIS - THEIR ALTERATION BY ANTI-CARCINOGENIC AGENTS - ANTIOXIDANTS

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Animal treatment with various antioxidants is known to inhibit tumorigenesis induced by benzo (a) pyrene (BP) and other carcinogens. We studied effects of butylated hydroxyanisole (BHA) on in vivo binding of BP to DNA and protein. BHA treatment of mice given BP resulted in decreases in total DNA binding by 21 and 32 percent, respectively, in lung and liver. The extent of reduction in total DNA-binding did not appear to correlate with inhibition of tumor formation. Analysis of the effect of BHA treatment on the nature of BP-DNA adducts showed that the adducts were affected to different extents. Amounts of (plus)-BP-7 beta, 8 alpha-diol-9 alpha, 10 alpha-epoxide-DNA (BPDE-I) adduct formed were decreased 56 percent in lung, while amounts of adducts due to recycled BP phenols, quinones and BP 4,5-oxide were unaltered. Thus, specific reduction in BPDE-I adduct appears to correlate with a 53 percent inhibition of pulmonary adenoma formation. Antioxidants appear to inhibit tumor formation in lung by specifically inhibiting amount of BPDE-I adduct formed. In rats predominant BP-DNA adduct(s) formed in lung and liver resulted from interaction of a recycled-BP phenol with DNA. Only small amounts of BPDE-I adduct were formed. Treatment of rats with BHA increased total DNA binding and amounts of all DNA-adducts formed.

63. INHIBITION OF THE NEOPLASIA OF THE LARGE BOWEL

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This proposal has two major objectives. The first is to find effective inhibitors of carcinogen-induced neoplasia of the large bowel. There are two components to this objective, one relating to direct acting carcinogens and the other to carcinogens requiring activation. A proposed strategy for inhibiting direct acting carcinogens occuring within the lumen of the large bowel is by use of high molecular weight inhibitors which are not absorbed or are poorly absorbed from the gastrointestinal tract. Two such groups of inhibitors are to be investigated. One group is bis-ditert-butyl phenol substituted mellitenes, and the other consists of compounds containing multiple phenolic functions on a mesitylene or benzene ring. Studies of the effects of the test compounds on neoplasia of the large bowel from N-methyl-N-nitrosourea administered intrarectally to rodents will be used as an initial experimental model. Work on inhibition of carcinogens activated in tissues will focus initially on the large bowel carcinogen methylazoxymethanol (MAM). The neoplastic effects of MAM are inhibited by butylated hydroxyanisole (BHA). Studies of compounds related to BHA are proposed to determine the relationship between chemical structure and inhibitory activity. Pertinent to this endeavor will be a determination of their effects on NAD(plus)-dependent alcohol dehydrogenase, a putative activating system for MAM. The second major objective of the present proposal is to identify compounds currently in the environment that have the capacity to inhibit large bowel carcinogens. Major emphasis will be placed on naturally occurring phenols and coumarins.

64. CHEMOPREVENTION OF DMH-INDUCED COLORECTAL NEOPLASMS

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It is necessary to identify agents which are capable of inhibiting carcinogenesis in the large bowel. Investigation into the mechanisms of colorectal carcinogenesis suggests that antioxidants may have such capability. Butylated hydroxyanisole (BHA) retards experimental large bowel carcinogenesis; ascorbic acid (AA) ingestion is associated with a reduction in number and size of adenomas in patients with multiple polyposis. Human consumption of both of these agents has proven to be well tolerated. Toxicity studies have not demonstrated significant problems.

The proposed study is designed to demonstrate efficacy of AA and BHA given concurrently in chemoprevention of experimental large bowel cancer. The optimum dose of each agent is to be determined. Effects of AA and BHA in combination will be studied. Toxicity will be documented.

First, AA and/or BHA will be administered orally or rectally in varying dosage to CF1 mice that are being given the large bowel carcinogen, 1,2-dimethylhydrazine (DMH). After 40 weeks, survival, tumor incidence and histology, and toxicity will be determined. Next, the procedure will be repeated in mice being fed only the minimum amount of food consumed by any group in experiment 1 in order to relate any growth effect or toxicity to dietary intake vs. toxicity of the drugs. Third, the most effective dose each agent and combination, as determined in experiment 1, will be administered to mice receiving DMH. Mice will be sacrificed at intervals to determine effect of AA and/or BHA on rate of development of large bowel neoplasm.

65. PURINE DERIVATIVES AND OTHER CHEMICAL CARCINOGENS

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In this laboratory the food additive (antioxidant) butylated hydroxytoluene (BHT) has been shown to inhibit the carcinogenic action of 3-hydroxyxanthine in rats by about 80 percent. This finding needs to be extended and other antioxidants tested for their suppression of carcinogenesis in the rat carcinogenesis assay. The effect of such compounds on the metabolism and intracellular distribution of 3-hydroxyxanthine will be studied. A possible effect of the carcinogen, as well as the anticarcinogen, on the level of cellular and urinary cyclic guanosine monophosphate will be investigated in collaboration with Dr. Ronald Coffey of this Institute. Carcinogens other than purine N-oxides are to be included in this investigation and integrated with combination assays of carcinogens and antioxidants in rat assays. 8-Methyl-3-hydroxyxanthine has been proved to have a uniquely interesting mode of metabolic activation. Its sulfate ester yields an intermediate which does not react electrophilically with any of the cellular nucleophiles with which 3-hydroxyxanthine reacts. Instead, it forms an oxidizing species with properties of a free radical. Its carcinogenic activity is much less than that of related strong carcinogens but it is not zero. This purine derivative will be studied as a example of a weak carcinogen with possible relevance to the action of weak environmental carcinogens. The studies will include reassay with a larger number of rats, accompanied by further metabolic studies.

66. INHIBITION OF CHEMICAL CARCINOGENESIS

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The ultimate goal of this program is to obtain data which can be used to determine the present or potential role of inhibitors of environmental chemical carcinogens in preventing neoplasia. Pursuant to this goal are several major objectives. The first is to identify inhibitors of chemical carcinogenesis. The second is to determine the mechanisms of inhibition by compounds having an inhibitory effect. The third is to determine chemical characteristics which control the ability of compounds to inhibit chemical carcinogenesis with the further goal of producing more effective inhibitors (higher potency and less toxicity). The fourth is to determine conditions under which inhibition occurs.

The first part of this renewal is a study of reactions between phenolic antioxidants such as butylated hydroxyanisole (BHA) and direct acting carcinogens. Efforts will be made to relate any such reactions to the capacity of these antioxidants to inhibit carcinogen-induced neoplasia. The second part focuses on two new groups of inhibitors, coumarins and aryl isothiocyanates. Efforts will be made to determine their mechanism of inhibition, relationships between chemical structure and inhibiting activity, and conditions under which inhibition occurs.

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67. SELENIUM SUPPLEMENT AND DIETARY FAT IN BREAST CANCER

Ip, C. C., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York,* 14263, United States of America

The objective of this research proposal is to determine the effect of dietary selenium supplementation on mammary carcinogenesis induced in rats by 7,12-dimethylbenz(a)anthracene (DMBA) with special emphasis on the ability of selenium to reduce the marked tumor incidence observed in rats fed a high fat diet. Our interest in selenium as a potential anticarcinogen stems primarily from its ability to protect against peroxidation. The lability of a given tissue to oxidative damage is a function of the balance between the concentration of polyunsaturated lipids and the antioxidant defense capacity. Thus uncontrolled lipid peroxidation may occur as a result of a high unsaturated fat intake and in the absence of an adequate supply of intracellular antioxidant. In this study, we will look into the effect of selenium status on the susceptibility of the mammary tissue to carcinogenesis and the lability of the tissue to lipid peroxidation in rats fed a high fat versus a low fat diet and in rats fed a saturated fat versus an unsaturated fat diet.

We are also interested in finding out whether additional vitamin E and methionine can enhance the efficacy of selenium in this regard, since these two compounds are known to potentiate the antioxidant status of the animal. In order to delineate if the action of selenium is distinct from other artificial antioxidants such as butylated hydroxytoluene (BHT), we will determine if the effects of selenium and BHT are additive in the inhibition of DMBA-induced mammary tumorigenesis. By supplementing the diet with selenium either before and after DMBA administration, we will also try to determine if selenium has any influence on DMBA metabolism. If we can conclusively demonstrate that low doses of selenium are effective in inhibiting the development of neoplastic diseases that are promoted by dietary fat, the use of selenium at nutritional levels can offer a potential means of cancer prevention.

68. MAMMARY CARCINOGENESIS - DIETARY FATS AND ANTIOXIDANTS

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An investigation of the influence of the amount and degree of unsaturation of dietary fat on the uptake and binding of 7,12-dimethylbenz(alpha)anthracene (DMBA) in mammary gland parenchymal cells and mammary fat pad cells, and how the presence or absence of antioxidant affects these parameters and tumor incidence is proposed. Preliminary studies indicate that dietary polyunsaturated fat is more antagonistic to the inhibitory effect of antioxidants on tumorigenesis than either a saturated fat or low fat diet, possibly due to the high peroxidative capabilities of unsaturated fats. Binding of labelled DMBA to the mammary parenchymal cell, DNA, RNA and proteins at various time intervals after DMBA administration will be determined in animals in the various dietary states and with or without antioxidant. Total DNA, RNA and protein of the isolated mammary parenchymal cells from animals on the various diets will also be determined. This information will be correlated with the uptake and binding of DMBA by the cells as well as the antioxidant content of the cells at various time intervals after DMBA administration and in the different dietary states. The studies described in this project are thus designed to probe the interrelations of antioxidants and dietary fat level and type on the uptake, storage and binding of DMBA at the level of the mammary gland parenchymal cell and fat pad cell.

69. BIOLOGY OF MAMMARY PRENEOPLASIAS

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The objectives of this research program are an analysis of the pathogenesis of murine mammary cancer using several model systems recently developed. The emphasis of the current research is on the inhibition of mammary tumorigenesis by Selenium in both viral and chemical carcionogen-induced mammary tumors. Experiments will be perfomed to determine the stages of mammary tumorigenesis which are sensitive to inhibition by Selenium. The second area of research to be pursued will be the development of appropriate in vitro cell culture conditions to analyze the effects of inhibitory compounds (i.e., Selenium) on the growth of normal, preneoplastic and neoplastic mammary cells.

70. FREE RADICALS IN NITROSO-UNSATURATED LIPID REACTIONS

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The amino nitrogen of the arylamine carcinogen 2-acetylaminofluorene (AAF) must be oxidized into the N-hydroxy compound (N-OH-AAF) in order to be a more potent form of the carcinogen. 2-Nitrosofluorene (NOF) can be formed from N-OH-AAF by 2 routes: 1) deacylation of N-OH-AAF and subsequent oxidation of N-hydroxy-2-aminofluorene and 2) by the free radical activation route which involves the nitroxyl free radical of N-OH-AAF. We have discovered a new reaction which involves 2-nitrosofluorene (NOF) addition to carbon-carbon double bonds of lipid molecules to form a free radical which we postulate to be the nitroxyl free radical form of NOF covalently bound to the unsaturated lipid molecule. The proposed chemical nature of the product of this reaction has a structure very similar to a very potent form of the carcinogen. NOF will not add to saturated lipids and in peroxidizing lipids, the rate of the reaction as well as the products of the reaction are influenced by antioxidants. Therefore, we believe our observations are pertinent to the general observations that polyunsaturated fats enhance and antioxidants depress carcinogenesis.

We propose to elucidate the molecular events in the reaction of NOF with oleic acid (one double bond) and determine if the product is mutagenic in the Ames mutagenic tester strains of Salmonella; and if it is found to be mutagenic then determine its carcinogenicity. We also propose to determine the orientation of the product in membranes and whether this product is transferred from the membrane to DNA. We plan to determine if the product reacts with DNA and if this reaction is influenced by antioxidants. We also plan to determine if NOF, when it interacts with nuclear membranes, yields the free radical observed in model membranes and if the rate of formation of the free radical or its stability are influenced by antioxidants.

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71. THE METABOLIC ACTIVATION OF POLYCYCLIC HYDROCARBONS IN CARCINOGENESIS - ASBESTOS-ENHANCED CARCINOGENESIS

O'Brien, P. J., Memorial University of Newfoundland, Dept. of Biochemistry, *Health Science Complex, St. Johns, Newfoundland,* A1C 5S7, Canada

The summary for this project is under entry no. 4.

72. ROLE OF ANTIOXIDANTS IN INHIBITION OF CARCINOGENESIS

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A number of procarcinogens undergo metabolic activation by the microsome mixed function of oxidase system. These include the polycyclic aromatic hycrocarbons of which benzpyrene has been the most extensively studied prototype - being converted to its intermediate or ultimate carcinogenic form by expoxidation through the NADPH dependent cytochrome P450 mixed function oxidases (MFO). In this laboratory we are presently studying a number of agents which alter the MFO activity in vivo. Their effect on conversion of benzpyrene to its epoxides is monitored in vitro in liver microsomes and liver explant systems and correlated with the effect of these agents in the Ames assay.

73. ANTIOXIDANTS INTERACTION WITH BENZOPYRENE & DERIVATIVES

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Benzo(a)pyrene (BP) is an environmental chemical carcinogen; the possibility of reducing the harmful effects of BP has been suggested by the observation that certain antioxidants inhibit the carcinogenesis of BP in test animals. It is the purpose of this research to gain a further understanding of the inhibitory effect of antioxidants on the mutagenicity and/or carcinogenicity of BP. The following specific questions are being addressed: (1) Do antioxidants affect the in vitro metabolism or mutagenicity of BP? If so, (2) do different types of antioxidants act similarly or differently, and (3) what specific effects do the antioxidants exhibit and what conclusions can be drawn regarding their mechanism of action?

These questions are being investigated by studying the effect of antioxidants on a) the in vitro metabolism of BP and some alkylated derivatives by rat liver microsomes, b) the mutagenicity of BP and derivatives towards the histidine dependent strains of Salmonella typhimurium (the Ames test).

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C. INHIBITION BY OTHER CHEMICAL AGENTS

74. INHIBITION OF CARCINOGENIC ACTIVITY OF AROMATIC AMINES BY INHIBITION OF THEIR N-O-SULFATION

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OBJECTIVE: One of the ultimate carcinogenic metabolites of several aromatic amines is often assumed to be the N-O-sulfate conjugate (Miller, Cancer Res. 38, 1479, 1978). We want to inhibit the formation of the N-O-sulfate by selective inhibition of sulfation, and to evaluate the potential preventive action of such inhibition on carcinogenesis by aromatic amines.

APPROACH: Two inhibitors of sulfation in vivo (Mulder and Scholtens, Biochem. J. 165, 553, 1977) will be used in the rat to study: 1) their effect on the metabolism of N-hydroxy-2-acetyl-aminofluorene; 2) their effect on the formation of acetylaminofluorene-adducts to macromolecules in the rat liver; and 3) their effect on carcinogenesis of this compound and related carcinogens.

PROGRESS: Both sulfation inhibitors, pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol (DCNP), strongly increased the formation of the N-O-glucuronide of N-hydroxy-2-AAF, and decreased the excretion of low-molecular-weight adducts of N-hydroxy-2-AAF, presumably glutathione conjugates, by the same amount. When the metabolism of N-hydroxy-2-AAF was studied in the perfused rat liver, omission of sulfate from the perfusion medium gave the same results as addition of PCP. PCP had the same effects in the rat in vivo and in the liver perfusion. It decreased the amount of N-hydroxy-2-AAF that became covalently bound to protein, RNA and DNA (Meerman, Van Doorn and Mulder, Cancer Res. 40, 3772, 1980). Specifically, the formation of N-acetylated adducts to DNA was inhibited by PCP, while the de-acetylated adducts were unaffected. PCP and DCNP prevent the hepatotoxic action of N-hydroxy-2-AAF.

75. INHIBITION OF CHEMICAL (LUNG) CARCINOGENESIS BY NAPHTHOQUINONE DERIVATIVES - IN VIVO AND IN VITRO STUDIES

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Feeding inhibitors of polycyclic aromatic hydrocarbon (PAH) metabolism resulted in an approximately 50% inhibition of pulmonary adenoma formation by 7,12-dimethylbenzanthracene (DMBA) in A/J mice. Two inhibitors were used, menadione glutathione adduct (MGA, thiodione), which inhibits both enzymatic 6-hydroxylation of benzo(a)pyrene (BP) and the aryl hydrocarbon (AHH) system, and benzylimidazol (BL) which is a preferential inhibitor of the more common ring hydroxylating microsomal P-450 (AHH) system but without effect on 6-hydroxylation. The relevance of the unique enzymatic 6-hydroxylation to pulmonary carcinogenesis is quite unknown and needs further clarification: (a) the 6-hydroxylase is essentially a lung enzyme, (b) during the non-enzymatic oxidation of the 6-hydroxy compound to diones, free radicals are generated and (c) the generation of free radicals results in the binding of the 6-hydroxy-compound to cellular constitutents including DNA; indeed tissue culture cell transformation has been accomplished by this derivative.

Therefore, this proposal explores an alternate mechanism to the study of AHH, an enzyme system which now appears unlikely to be the sole determinant of lung tumorigenesis, although it may be the most important. Our studies concern the inhibition of chemically induced lung carcinogenesis in vivo using a variety of inbred strains of mice.

We also wish to perform in vitro studies dealing with the 6-hydroxylase system and the inhibition of chemical carcinogenesis by naphthoquinone derivatives.

76. ROLE OF CYCLIC NUCLEOTIDES IN TUMOR PROMOTION

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Our major objective is to elucidate the mechanisms of tumor promotion, which appears to be a component of carcinogenesis in general, and its inhibition by various agents, with special reference to the role of cyclic nucleotides. We have shown that the promoter phorbol myristate acetate (PMA) produced a marked and prolonged stimulation of cyclic GMP synthesis in mouse epidermis. We will study: 1) The mechanisms responsible for this stimulation, which may involve release of unsaturated fatty acids and increased prostaglandin synthesis, and 2) the possible effects of increased cGMP, such as changes in chromosomal protein phosphorylation. We have shown that butyric acid inhibits promotion, induces beta-adrenergic hormone receptor synthesis and facilitates the coupling of beta-receptors to adenyl cyclase in mouse epidermis. The uncoupling effect of PMA is overcome by butyric acid. The role of uncoupling in tumor promotion will be clarified by testing other uncoupling agents such as filipin and amphotericin B as promoters. The mechanism of butyric acid inhibition of promotion may involve its effect on the coupling reaction or on altered histone acetylation which will also be examined. Agents which inhibit cGMP stimulation and protein phosphorylation will be tested as promotion inhibitors.

77. THE ENHANCEMENT OR INHIBITION OF CARCINOGENESIS AND MUTAGENESIS BY REDUCING AGENTS

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OBJECTIVE: To develop a short-term in vitro assay to identify agents which modify the mutagenic/carcinogenic activity of carcinogens.

APPROACH: Logarithmically growing cultures of Salmonella typhimurium are suspended in treatment mixtures consisting of known carcinogens and test agents such as reducing compounds. A measurement is made of the influence of the reducing agents on the frequency of reversion to histidine prototrophy of treated cultures.

PROGRESS: Studies have been made on the effect of a range of reducing agents and food additives on the mutagenic activities of several carcinogens. We have found that a reducing agent such as cysteine is able to inhibit the mutagenic action of an array of carcinogens (N-methyl-N'-nitro-N-nitrosoguanidine or MNNG, methyl methane sulfonate, 4-nitroquinoline-1-oxide or 4-NQO, N-acetoxy-2-acetylaminofluorene or N-acetoxy-AAF, N-hydroxy-2-acetylamino-fluorene or N-hydroxy-AAF, aflatoxin B1, 5-nitro-2-furaldehyde semicarbazone, 2-(2-furyl)-3-(5-nitro-2-furyl)) acrylamide, and the nitrosation products of methylurea and methylguanidine. Antioxidants displayed different abilities in inhibiting the mutagenic actions of carcinogens. Bisulfite ascorbate and disulfiram inhibited MNNG- but not N-acetoxy-AAF-induced mutagenesis, whereas butylated hydroxyanisole and alpha-tocopherol did not affect the mutagenic activity of either carcinogen. The assay also indicated the ability of some agents to enhance a carcinogen's mutagenic action. The food additive propyl gallate increased the mutagenic activities of 4-NQO and the proximate carcinogen N-hydroxy-AAF. The same food additive decreased the mutagenic action of MNNG, N-acetoxy-AAF and aflatoxin B1. These data indicate the complexity of studies made on carcinogen-modifying agent interactions and support the further use of short-term assays to obtain a better understanding of such interactions.

78. NICKEL CARCINOGENESIS

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The long-term objective of this research program is to elucidate the mechanisms of carcinogenesis by nickel compounds. The specific aims of this proposal are: (a) to investigate the inhibitory effect of manganese upon the carcinogenicity of nickel subsulfide (alpha-Ni3S2); (b) to identify the metabolic basis for the pronounced differences which have been found between the carcinogenicities of alpha-Ni3S2 and nickel sulfide (NiS); and (c) to characterize and standardize an experimental system for induction of renal carcinomas in rats by intrarenal injection of alpha-Ni3S2. The methods include: (a) carcinogenesis testing following administration of alpha-Ni3S2, NiS, Mn and other compounds to Fischer rats by intramuscular and/or intrarenal injection; (b) histological and electron microscopic investigations of pathological lesions that are associated with induction of renal carcinomas by alpha-Ni3S2; and (c) compartmental analysis of 63Ni and 35S kinetics in order to identify differences in the metabolism of radiolabelled alpha-Ni3S2 and NiS in rats. This study is providing fundamental knowledge of metal carcinogenesis and is also furnishing information which has practical applications to assessing human hazards of carcinogenesis from nickel compounds, with particular relevance to energy research, industrial toxicology and environmental pollution.

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79. INHIBITION OF TUMOR PROMOTION BY PROTEASE INHIBITORS

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The major cause of cancer appears to be the environment. Estimates of 80-90% of cancer incidence have been ascribed to environmental factors. Carcinogenesis is a muitistep process which can be conceptually subdivided into initiation and promotion areas. Cancer could be prevented by avoiding exposure to initiating carcinogens or by interfering with the biological processes leading to neoplastic transformation. We propose to prevent cancer by modifying the biological response with protease inhibitors. A novel application in the use of protease inhibitors is in feeding diets containing natural protease inhibitors e.g., soybeans. Protease inhibitors (antipain, tosyllysine chloromethyl ketone (TLCK) have been shown to block error-prone repair of DNA. TLCK, Tosyllysine phenylalanine chloromethyl ketone (TPCK) and leupeptin applied to skin and soybean trypsin inhibitors in the diet have been shown to interfere with promotion by the tumor promoter 12-O-Tetradecanoyl-phorbol-13-Acetate (TPA). Protease inhibitors in the diet have suppressed breast cancer response in Sprague-Dawley rats. The high occurrence of protease inhibitors in plants may be responsible for the low cancer occurrence in vegetarian populations. The Seventh-Day Adventists exhibit low breast and colon cancer--cancers whose occurrence are not related to cigarette smoking. To support this thesis, we will feed protease inhibitors extracted from plant products used in human nutrition, e.g., beans, peanuts, corn and potatoes to animals subjected to carcinogenesis and compare the tumor yield to those fed a diet free of protease inhibitors. The animal systems which will be used are two-stage carcinogenesis in the mouse, breast and bladder cancer in the rat. Two protease inhibitors from the soybean have been purified and extensively studied--the Kunitz inhibitor MW 21.500 and the Bowman-Birk inhibitor MW 21.500 and the Bowman-Birk inhibitor MW 8000. We will study the digestion of these inhibitors by pepsin and absorption of radioactively labeled inhibitors or its digestion products on feeding to animals. The heat stable Bowman-Birk inhibitor has been shown to yield smaller fragments still capable of inhibiting proteases. The occurrence of protease inhibitors in human food materials (cereals, Bread, cooked beans, peanuts) will be investigated, in order to identify foods with (Text Truncated - Exceeds Capacity)

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80. THE ROLE OF PROMOTERS IN TWO-STEP CARCINOGENESIS

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OBJECTIVE: To attempt to correlate aberrant chromosomal segregation events with transformation following chemical carcinogen treatment, by the use of known inhibitors of promotion and anticarcinogenic agents.

APPROACH: V79 Chinese hamster lung fibroblasts are treated either with carcinogen alone or simultaneously with the carcinogen and inhibitor, in the presence of 3 times 10 to the minus 5th power M BUdR for twenty-seven hours or two cell cycles. A comparison is then made of the sister chromatid exchange (SCE) frequencies and aberration frequencies in the presence or absence of the inhibitor.

In addition, these studies are being correlated with the effects of inhibitors of promotion on chemically induced transformation, in the C3H 10T 1/2 mouse transformation system.

PROGRESS: Antipain, an anti-protease and inhibitor of promotion inhibits N-methyl-N'-nitro-N-nitrosoguanindine (MNNG)-induced chromosome aberrations and inhibits MNNG-induced transformation (Kinsella and Radman, in press).

81. INHIBITION OF MUTAGENESIS AND CARCINOGENESIS BY PROTEASE INHIBITORS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

82. STUDIES OF NATURAL INHIBITORS OF CHEMICAL CARCINOGENS

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OBJECTIVES: To evaluate a number of naturally occurring substances which have been reported to have anti-carcinogenic activity. To determine their identity, structure and mode of action.

WORK SCOPE: Employ the rainbow trout-aflatoxin B1 model for induction and development of hepatocellular carcinoma as a test system for detection of substances in the environment which may modify the carcinogenic response. Conduct such in vivo assays as indicated to explain modifying actions of the natural substances employed on aflatoxin metabolism. The formation of aflatoxins P1 and M1 is of particular interest. Perform gross pathology and histopathology (on questionable lesions), and histopathology on a subset of all the tumors. Perform enzyme assays in selected instances for aldrin epoxidase, styrene epoxide hydrase, O-dealkylase, GSH-S-arenetransferase, and other enzyme systems as indicated. Characterize certain vegetable materials in the trout liver carcinoma model for the capacity to inhibit the carcinogenic process; such vegetables may include all of the Brassicaceae family. Characterize for inhibiting capacity certain defined chemical compounds such as benzyl thiocyanate and phenethyl isothiocyanate, flavones, methoxyflavones, tangeretin, nobilitum B-ionone, selenium compounds and others. For these inhibition studies, test indicated substances and compounds at two levels in the diet using the Oregon Test Diet and adjusting diets so that they are isonitrogenous and isocaloric.

83. MICROSOMAL INDUCTION AND RESPONSE TO CARCINOGENS

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The overall objectives of the present research proposal are: (1) To identify naturally occurring compounds that have the capacity to inhibit chemical carcinogenesis, (2) to establish conditions under which inhibition occurs, and (3) to determine the mechanism or mechanisms by which observed inhibitory effects are brought about. The principal focus with regard to investigations of mechanisms of inhibition will be on naturally occurring indoles found in cruciferous plants. These compounds inhibit polycyclic aromatic hydrocarbon-induced neoplasia in rodents. Studies of their effects on polycyclic aromatic hydrocarbon metabolism will be undertaken in an effort to elucidate the alterations which are responsible for inhibition of carcinogenesis. Additional investigations will be carried out to determine if plants other than Cruciferae have constituents which alter carcinogen metabolism so as to increase detoxification. If so, attempts will be made to isolate and identify the responsible constituents.

84. STUDY OF NATURAL INHIBITORS OF CHEMICAL CARCINOGENS

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As an important adjunct to the testing of crude natural materials or defined chemicals for their inhibitory capacities, studies of the effects of test substances on carcinogen metabolism will be carried out. This will serve several purposes. In the case of crude materials, it will provide a potential assay for use in isolating and identifying the inhibitor. For both crude and defined test substances it may provide corrobative evidence for in vivo inhibition and also might provide some information on mechanism of inhibition. In some instances, it may detect weak or unusual effects that merit further exploration. Ultimately the metabolism studies may be developed into preliminary screening techniques useful in selecting materials for study in full animal carcinogeesis testing.

85. ENDOGENOUS AND EXOGENOUS FACTORS IN CHEMICAL CARCINOGENESIS

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The effects that structural alterations may have on the carcinogenicity or toxicity of various compounds are determined. Methods to inhibit the action of known carcinogens by administration of other compounds are devised. The biochemical, morphological, and physiological bases for such inhibitory effects are studied.

BIBLIOGRAPHIC REFERENCES: Evarts, R. P., Brown, C. A. and Atta, G. J.: The effect of hydroxylamine on the morphology of the rat mammary gland and on the induction of mammary tumors by 7,12-dimethylbenz(a)anthracene. Exp. Molec. Pathol. 30: 337-348, 1979. Mostafa, M. H. and Weisburger, E. K.: Effect of chlorpromazine hydrochloride on carcinogen metabolizing enzymes: Liver microsomal dimethylnitrosamine demethylase, 4-dimethylaminoazobenzene reductase and aryl hydrocarbon hydroxylase. J. Natl. Cancer Inst. 64: 925-929, 1980.

III. MODIFICATION OF CHEMICAL CARCINOGENESIS IN MODEL SYSTEMS BY HOST FACTORS

A. ROLE OF IMMUNE STATUS IN CHEMICAL CARCINOGENESIS

86. PROTECTIVE IMMUNITY TO CHEMICAL CARCINOGENS

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Our principal research objective is to determine whether it may be feasible to protect experimental animals from tumors induced by polycyclic aromatic hydrocarbon (PAH) carcinogens by immunizing them with non-carcinogenic analogs of these carcinogens. In previous work, we have shown that one such analog, 5-FMBAAA, can induce antibodies capable of neutralizing PAH carcinogens in tissue culture and reducing the body burden of these carcinogens in mice exposed to the carcinogens in vivo. Our current experiments are designed to determine whether this type of immunization is actually protective against carcinogenesis per se - i.e., does it reduce the incidence of tumors induced in mice by exposure to PAH carcinogens? Male CD-1 mice are immunized with 5-FMBAAA conjugated to bovine serum albumin in order to render it antigenic. These mice, along with control mice, are then subjected to repeated skin applications of the PAH carcinogen, dimethylbenzanthracene (DMBA), alternating with the promoter, PMA. Tumor incidence is recorded weekly to determine whether the immunization prevents the induction of tumors by the DMBA-PMA regimen.

BIBLIOGRAPHIC REFERENCES: Moolten, F., and Schreiber, B.: Formation of Adherent Monolayers of Murine Lymphocytes In Vitro. J. Immunol. Methods, 1980, in press. Moolten, F., Schreiber, B., Rizzone, A., Weiss, A., and Boger, E.: Immunization Against Polynuclear Aromatic Hydrocarbon Carcinogens. Proc. of Battelle Symposium on Polynuclear Aromatic Hydrocarbons, 1981, in press.

87. INITIATION-PROMOTION CARCINOGENESIS - ROLE OF ANTIBODY

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The objective of the proposed investigation is to determine if autoantibodies play a role in tumor promotion. The model for study is two stage skin carcinogenesis in mice using 7, 12-dimethylbenzanthracene (DMBA) as the carcinogen and 12-O-tetradecanoylphorbol-13-acetate (TPA) as the promoting agent. The hypothesis of the investigation suggests that TPA promotes

the DMBA-initiated tumors by induction of autoantibody and it is the antibody that stimulates the tumor cells. Support for the hypothesis has been obtained by demonstration in our laboratry of 1) the in vitro stimulation of tumor cell division by antiserum prepared to normal cells from which the tumor arose, 2) the detection of antibodies to skin in mice treated on the skin with TPA and 3) the production of isologous skin autoantibodies in mice, 4) the promotion of DMBA-initiated skin tumors by injections of isologous skin.

If autoantibody is responsible for promotion by TPA then it should be possible to demonstrate promotion by the spleen cells from TPA-treated mice. In the proposed experiments, spleen cells from TPA-treated mice are to be adoptively transferred to DMBA-initiated mice in several different ways. The spleen cells from TPA-treated mice are also studied in vitro for their ability to promote or stimulate tumor cells.

88. GENETIC CONTROL OF IMMUNE RESPONSE TO METHYLCHOLANTHRENE-INDUCED MURINE FIBROSARCOMAS

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OBJECTIVE: To study whether the immune response to individual tumor-associated antigens (TATA) of methylcholanthrene (MCA)-induced murine sarcomas is under control of genes of the major histocompatibility complex.

APPROACH: MCA-induced, TATA-bearing sarcomas of various inbred strains of mice will be used to evaluate the anti-TATA immune response of the strain of origin of the tumor in comparison with the immune response of different F1 semi-syngeneic hybrids. The immune response will be evaluated both in vivo (growth, excision and challenge method) and in vitro (CML or 51Cr labelled tumor cells). Possible linkage between high or low immune responders and H-2 haplotype will be studied by backcross tests.

PROGRESS: Evidence for a polygenic control of the in vivo immunity to TATA of two BALB/c sarcomas has been obtained, involving both non-H-2 genes and products of the MHC.

89. CARCINOGEN INDUCED FACILITATION OF TUMOR GROWTH

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A possible mechanism for interaction of environmental carcinogens such as hydrocarbon emissions may be specific or non-specific deleterious effects on immunoregulation during chemical carcinogenesis.

Treatment of C3H mice with 1.0 or 0.05 mg of 3-methylcholanthrene (3-MC) has been found to result in acceleration of growth of transplanted syngeneic fibrosarcomas. The purpose of this study is to further investigate the immunobiology of 3-MC induced facilitation of syngeneic tumor growth. The study will determine if the effect is (a) due to immunosuppression, (b) transferable to a syngeneic recipient, (c) immunologically mediated, (d) a general phenomenon or demonstrable with only occasional tumors, and (e) observed during chronic administration of split doses of carcinogen.

The study will determine the effect of 3-MC on the hemolysin plaque assay, skin graft rejection, lymphocyte mitogen stimulation, in vitro tumor cytotoxicity, macrophage phagocytic function, and mixed lymphocyte culture.

To test whether carcinogen induced acceleration of tumor growth is transferable to syngeneic recipients and immunologically mediated, the techniques of parabiosis, passive transfer of spleen and lymph node cells, and passive transfer of serum will be used. In addition, the effect of carcinogen on second set tumor rejection will be investigated.

90. ROLE OF THE IMMUNE SYSTEM IN EXPERIMENTAL CHEMICAL TOXICITY AND CARCINOGENESIS

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This program was initiated in FY 1976 to study basic immunofunction in animals during recovery from chemical insults and preceding and during carcinogenesis so that the immune system may be assessed in an intelligent fashion as a prophylactic or therapeutic measure in dealing with such health hazards to the human population. An animal model was developed that would parallel the human situation and provide a wide spectrum of information. Techniques with the rat and hamster have proven successful, and appropriate tests can now be performed with lymphocytes from spleen, thymus, and blood. Nine tests can be used on a single animal to detect perturbations in specific lymphocyte number, functions, and interactions. The tests will be applied to aromatic hydrocarbon-induced carcinogenesis of the respiratory tract (benzo(a)pyrene) and chemically induced (nitrosamines, dimethylhydrazine) carcinogenesis of the gastrointestinal tract. Carcinogenic properties of aromatic hydrocarbons alone and in combination with other potentially synergistic agents in immunologically deprived animals will be determined. Oil shale extraction by-products to be studied include polycyclic aromatics, organic amines, thioethers, organic acids, esters, phenols, organo-metallic compounds, alkanes, alkenes, and potentially toxic trace metals.

91. THE SENSITIVITY OF NUDE MICE TO CHEMICAL CARCINOGENS

Elgjo, K., Iversen, O. H., Universitetet i Oslo, Inst. of Pathology, *Rikshospitalet, Oslo 1, Norway*

OBJECTIVE: To determine whether the immunodeficiency of nude mice leads to a higher skin tumor yield after topical surface applications of carcinogens.

APPROACH: Nude mice and a control group of BALB/c mice are painted in single weekly applications with DMBA. The yield of papillomas and malignant tumors is observed. The mice are kept under specific pathogen-free conditions.

PROGRESS: Up to now, many papillomas have appeared on the BALB mice, and no papillomas on the nude mice. It thus seems that nude mice are more resistant to carcinogens than normal mice. However, there may be many explanations for this phenomenon. The investigation continues.

92. ENHANCEMENT OF NBU-LEUKEMOGENESIS IN MICE BY INJECTION OF THYMUS CELLS

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OBJECTIVE: To study the effect of thymus cells in N-nitrosobutylurea(NBU) leukemogenesis in a mouse system.

APPROACH: C57BL/6J, CBA/H-T6(T6), AKR/J and its syngeneic substrain AKR/T1ALD(T1) mice were used. T6 and T1 mice have marker chromosomes. 1) Thymectomized (C57BL X T6)F1 mice were treated with NBU after either intraperitoneal injection of parental C57BL thymus cells or intraperitoneal insertion of thymus tissues in a diffusion chamber. 2) Sublethally irradiated AKR mice were treated with NBU after restoration by intravenous injection of (AKR X0T1)F1 spleen or bone marrow cells mixing with or without thymus cells of T1 mice. It is possible to determine the origin of the leukemic cells by marker chromosome. T-cell characteristics of leukemic cells were ascertained by the cytotoxicity test for theta-antigen.

PROGRESS: 1) An increased incidence of leukemia was observed in thymectomized mice only after thymus cells injection. The thymus in diffusion chamber has no effects on the development of leukemias. All the leukemias originated from host cells and have no theta-antigen on their cell surface. 2) A marked acceleration of leukemia development was noticed in irradiated mice which restored by cell injection mixing with thymus cells. In this particular system, leukemic cells originated from donor spleen or bone marrow cells, but not from injected thymus cells. Forty percent of thymus enlarged leukemias which restored by bone marrow cells were theta-negative whereas the majority of leukemias which occured in restored spleen mice or bone marrow with thymus cells restored mice were theta-positive. Further, our chromosomal analysis indicated that repopulation ability of spleen and bone marrow cells into the thymus can be accelarated by mixing with thymus cells in inocula.

These results suggest that thymus cells are not target cells of NBU and may somehow have influence on cell kinetics of lymphoid precursor cells in the spleen or bone marrow probably by cell to cell interaction.

93. IMMUNOBIOLOGY OF TRANSPLACENTAL CARCINOGENESIS

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The objective is to determine the role of immunocompetent cell dysfunction or lack of maturation that is associated with the high efficiency of transplacental carcinogenesis. We have observed that pregnant, but not other, Erythrocebus patas monkeys are immunosuppressed by the carcinogen, 1-ethyl-1-nitrosourea (ENU). Mice are resistant to ENU unless they are also injected with the tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA). We will determine if ENU synergizes with TPA and/or pregnant monkey serum to immunosuppress mouse lymphocytes in vitro and in vivo. Vitamin A acid (retinoic acid) abrogates the promoting effect of TPA and prevents induction of ornithine decarboxylase (OCD). OCD produces putrescine. We will test the effect of retinoic acid on the immunosuppressive effects of ENU with TPA and on transplacental carcinogenesis itself.

94. CARCINOGENICITY AND METABOLISM OF 5-NITROFURANS

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Studies will be conducted to identify and investigate the biochemical and immunological factors of probable importance in the carcinogenesis of experimental neoplasms induced in animals by 5-nitrofurans and related nitro-and amino-aromatic and heterocyclic compounds. Utilizing certain 14C-labeled nitrofuran carcinogens, the isolation and characterization of metabolic adducts to fragments of DNA, RNA, or protein will be studied. Investigations of alterations of humoral and cellular immunologic characteristics of animals chronically administered carcinogenic and non-carcinogenic nitrofurans will be conducted. The pathogenesis and prevention of neoplastic formation in rats, mice, and hamsters by selected compounds will be studied. Investigations of dosage effects, differential species and strains susceptibility, and additional structure-activity carcinogenesis relationships will be conducted. The objectives of these studies are to define and describe the experimental conditions under which 5-nitrofuran compounds and chemically related derivatives act as chemical carcinogens, and to identify and modify those factors of seeming importance in the development of tumors induced by these chemicals.

BIBLIOGRAPHIC REFERENCES: Takano, S., Matsushima, M., Erturk, E., and Bryan, G.T. Early induction of rat colonic epithelial ornithine and S-adenosyl-L-methionine decarboxylase activities by N-methyl-N-nitro-N-nitrosoguanidine or bile salts. Cancer Res., 41:624-628, 1981. Headley, D.B., Klopp, R.G., Michie, P.M., Erturk, E., and Bryan, G.T. Temporal comparisons of immune status and target organ histology in mice fed carcinogenic 5-nitrofurans and their nornitro analogues. Cancer Res., 41:(in press), 1981.

95. EFFECT OF C. PARVUM ON ACTIVATION OF MACROPHAGES AND ITS POSSIBLE ROLE IN DIMETHYL HYDRAZINE INDUCED COLONIC CANCER IN RATS

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OBJECTIVE: To determine what effect activation of the immune system has on the effluent cells from the gastrointestinal tract and to examine what effect such activation has on colonic cancer.

APPROACH: Corynebacterium parvum is known to activate the immune system particularly as far as macrophages are concerned. Such activation has been shown to have a beneficial effect on certain tumors, for example, mammary carcinoma. It is proposed to use the rat model (MacPherson and Steer 1979) to study the effect of C. parvum on this gut effluent. It is planned to induce colonic cancers in a group of rats by using dimethyl hydrazine. The gut effluent will be examine in these tumor-bearing animals before and after activation of the immune system with C. parvum.

BIBLIOGRAPHIC REFERENCES: MacPherson, G.G. and H.W. Steer (1979). Properties of mononuclear phagocytes derived from the small intestinal wall of rats. In function and structure of the Immune System ed. Mueller-Ruchholtz and Muller-Hermelink. pp. 433-438.

96. ENVIRONMENTAL CONTAMINANTS - EFFECTS ON TUMOR IMMUNITY

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The proposed study will determine the effects of lead and PCB (Aroclor 1254) on the immune response of mice to a growing tumor and the effects of contaminant-induced immune alteration on the incidence and growth rate of tumors. Several parameters of immunity will be assessed, including cell-mediated cytotoxicity, serum blocking activity, antibody-dependent cell-mediated cytotoxicity, and lymphokine production. Lead will be administered in the water at levels of 13, 130 or 1300 ppm PB. PCB will be administered in the feed at levels of 10, 100 and 500 ppm. Two tumor systems, the MSV-induced sarcoma and a MCA-induced tumor, will be used to compare the differences between regressive and progressive tumors.

97. SPECIFICITY OF T CELL-MEDIATED CYTOTOXIC REACTIONS WITH CHEMICALLY INDUCED MURINE SARCOMAS

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Chemically induced sarcomas of mice have antigens that engender specific anti-tumor immune response that can bring about rejection in preimmunized syngeneic recipients. In contrast to the individually distinct tumor antigens defined by transplantation techniques with these sarcomas, in vitro studies for cell-mediated immunity have sometimes suggested the presence of shared tumor-associated cell surface antigens. However, the lack of critical specificity controls for these in vitro reactions makes interpretation of these studies extremely difficult. The fact that MuLV and its antigens are often found in chemically induced sarcomas, and that normal mice frequently respond to these antigens, has added another serious complication to the study of these tumors. We propose to use in vitro cell-mediated cytotoxicity assays and in vivo Winn assays, as well as information accumulated through serological analysis of these sarcoma cells to define the cell surface antigens of chemically induced tumors that are recognized by cytotoxic T cells. Specificity of cytotoxicity will be analyzed by 1) cellular immunoadsorption tests with a series of syngeneic and allogeneic MuLV positive and MuLV negative fibroblast and sarcoma cell lines grown as monolayer cultures, 2) cellular inhibition assays which test the effects of MuLV positive and MuLV negative cell lines on cell-mediated cytotoxicity in vitro and in Winn assays in vivo, and 3) antibody blocking tests which measure the effects of high specific antibody to tumor-specific cell surface antigens (e.g., Meth A), alloantigens and MuLV-related antigens, on the interaction of cytotoxic T cells with target cells and on cellular adsorption of cytotoxic T cells.

98. CHANGES IN THE ANTIGEN STRUCTURE OF MOUSE EPIDERMIS DURING CARCINOGENESIS

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OBJECTIVE: To determine whether painting of hairless mouse skin with carcinogens leads to deletion of some of the epidermal antigens, and possible occurrence of new antigens.

APPROACH: Antibodies are produced by injection of homogenized epidermis, hyperplastic epidermis during carcinogenesis (papillomas and carcinomas) into rabbits. Immunological analysis is made by the gel diffusion precipitation line technique of Ouchterlony.

PROGRESS: It has been observed that during carcinogenesis, the epidermis loses some of its specific antigens, and acquires one new antigen which is also found in normal thymus and spleen, and probably comes from lymphocytes which are always present in papillomas and carcinomas.

99. IMMUNE SURVEILLANCE AND CANCER

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After completing extensive studies on spontaneous and induced tumors in nude mice, which showed no major differences in tumor incidence, type or biological behavior between the athymic nude mice and their controls, we plan to continue the work along two main lines: 1) to characterize possible thymus-independent surveillance mechanisms in the nude mouse to explain the 'normal' incidence of tumors and 2) to determinine if the natural cytotoxic cells detected in nude and normal mice in vitro, against targets derived from solid tumors (chemically induced, spontaneous, viral-induced mammary tumors, etc.) may have in vivo significance. These correlations will include attempts at in vivo abrogation of the thymus-independent putative surveillance mechanisms and determine the effects of such procedures on tumor development and biological behavior. To a large extent, this is a re-evaluation of the immune surveillance concept, but as a non-thymus-dependent proposition.

BIBLIOGRAPHIC REFERENCES: Stutman, O., Dien, P., Wisun, R., Lattime, E.C.: Natural cytotoxic (NC) cells against solid tumors in mice: Blocking of cytotoxicity by D-mannose. Proc. Natl. Acad. Sci (U.S.A.) 77: 2895-2898, 1980. Stutman, O.: Immunological surveillance and cancer, in The Handbook of Cancer Immunology, Vol. 7, H. Waters, ed. Garland STPM Press, New York, 1981, pp. 1-25.

100. BIOLOGY OF MAMMARY NEOPLASIA

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Elucidation of the biology of initiation and emergence of epithelial neoplasms, as illustrated by mammary and cervicovaginal tumorigenesis, is the overall objective of this proposal. Our research plan combines a variety of in vivo and in vitro experimental approaches to these problems, utilizing mouse and rat model systems and, in some cases, human cells. Experiments are designed to analyzed the cellular composition of the mammary gland and its tumors and to examine the roles of hormones, viruses, chemical agents, and cellular and host factors in the growth of normal and neoplastic cells, including metastatic tumors. Cervicovaginal studies with the mouse model will focus on dysplasias induced by perinatal steroid administration and their subsequent transformation into carcinomas in host environments modified by hormonal and immunological manipulations. Antigenicity and the metastatic behavior of these carcinomas will also be analyzed. We expect that our studies on these two systems will generate models that can be used for both in vitro and in vivo analyses of other epithelial neoplasms.

B. HORMONE-RELATED MODIFICATION OF CHEMICAL CARCINOGENESIS

101. MECHANISM(S) OF STEROID ACTION IN NORMAL MAMMARY GLAND AND IN 7,12-DIMETHYLBENZANTHRACENE (DMBA)-INDUCED MAMMARY TUMORS IN THE RAT

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OBJECTIVE: The objective of this project is to further understand the mechanism(s) of steroid action in normal mammary gland and in DMBA-induced mammary tumors and to compare the results between these two tissues.

APPROACH: Binding characteristics for estrogens, glucocorticoids, androgens and progestins will be studied in cytosol and nuclei as well as nuclear transfer of the steroid receptor complexes in normal mammary gland and in DMBA-induced mammary tumors. Moreover, hormonal factors controlling the levels of four classes of steroid receptors will be investigated in detail.

PROGRESS: The detailed characteristics of estrogens (3H) E2, progestins (3H) R5020, glucocorticoids (3H) Dex and androgens (3H) DHT binding have been determined by cytosol prepared from DMBA-induced mammary tumors. Exchange conditons have been determined for (3H) E2 and (3H) R5020 binding in DMBA cytosol. Moreover, since high levels of (3H) E2 and (3H) Dex binding are present in cytosol prepared from mammary gland of lactating rats, binding characteristics of these two steroids have been studied. Concerning the hormonal factors controlling steroid binding levels in cytosol prepared from DMBA-induced mammary tumors, we have clearly shown that following hypophysectomy or castration, (3H) R5020 binding levels are markedly decreased while (3H) Dex binding remains unchanged. Finally, the data obtained already indicate differences of steroid binding properties or levels in cytosol prepared from DMBA tumors and mammary gland of lactating animals.

102. ISOLATION AND CHARACTERIZATION OF PROLACTIN INHIBITING FACTOR (PIF) AND PROLACTIN RELEASING FACTOR (PRF)

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The existence of a prolactin inhibiting factor (PIF) and a prolactin releasing factor (PRF) is suggested by many experimental approaches (pituitary transplantation, pituitary stalk section, lesions of the hypothalamus, electrical stimulation of specific hypothalamic areas and infusion of hypothalamic extracts). Therefore, isolation and knowledge of the structure of these hypothalamic hormones appear to be of prime interest. In fact, the objective remission of breast cancer induced by L-dopa or ergot derivatives suggests a possible use of PIF or PRF antagonists in the therapy of breast cancer. Porcine hypothalami extracted will be subsequently purified by techniques based upon the molecular size of the polypeptides, their partitioning properties and their amphoteric characteristics. An assay using dispersed anterior pituitary cells in monolayer culture will be used to screen the biological inhibiting or stimulating activity of prolactin secretion in each fraction. The fractions active in vitro will be then assayed in in vivo tests specially designed for the hypothalamic hormone. TLC and TLE will be mainly used to determine the purity of the fractions.

After purification, isolation and elucidation of the structures of PIF and PRF, synthesis of the new molecules, and comparison of their biological activity against the natural molecules will be undertaken.

The effect of synthetic PIF and of PRF antagonist upon growth of breast tumor induced by 7,12-dimethylbenzanthracene (DMBA) would then be investigated. Studies of the inactivation and organ distribution of the synthetic peptides will also be performed in view of potential clinical use. The possible role of prolactin in breast cancer and the relatively high frequency of nonpuerperal galactorrhea indicate clearly the importance of gaining knowledge about the nature of the hypothalamic factors regulating PRL secretion. Knowledge of the structure of these substances should permit synthesis of compounds of clinical use.

103. PROLACTIN - ADRENAL INVOLVEMENT IN DMBA MAMMARY TUMORS

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A total of ten experiments have been performed during the period covered by this progress report, three basic and 7 DMBA tumor studies. The administration of corticosterone in the drinking water of intact rats blunted prolactin afternoon surges. Transplantation of adrenals to kidney capsule resulted in lower corticosterone levels but a diurnal rhythm in serum corticosterone was evident. Adrenalectomized (ADX) animals with dialysis sacs containing corticosterone survived a 30 day period in good health while animals bearing silastic capsules with a similar amount of corticosterone were dead by the end of the experimental period. Animals ADX at 45 days of age and placed on corticosterone in the drinking water had a delayed incidence of tumorigenesis with fewer tumors per animal. Animals ADX at 40 days of age were comparable to controls in their pattern of tumorigenesis. The administration of PRL prior to and after DMBA administration hastened mammary tumor formation. There was no difference in the expression of mamma-

ry tumorigenesis when DMBA was injected in the morning vs the afternoon; however, in those animals given DMBA in the afternoon that were in vaginal proestrus, the appearance of first tumor was delayed. Intact animals given corticosterone in the drinking water were observed to have tumors earlier than animals drinking tap water. Continued estrogen stimulation initiated either before or after DMBA administration blocked the incidence of mammary tumorigenesis.

104. MILK PROLACTIN BINDING AND THYROID IN BREAST CANCER

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In lactation, the liver synthesizes most of the carbohydrate and some of the lipid that is secreted in milk, and the liver as well as the mammary glands have receptors for prolactin. We have hypothesized that lactation may be regulated in part by a humoral negative feedback system, involving a factor in milk which diffuses from the breast into the plasma and causes inhibition of prolactin binding in the liver and mammary glands, thereby decreasing the response of these organs to prolactin. In investigating this hypothesis, we have found that the s.c. administration of bovine milk to female rats significantly decreases the capacity of the liver cell membranes to specifically bind 125I-ovine prolactin. We propose to: a) attempt to identify the prolactin-binding inhibiting factor (PBIF) in milk; b) examine its mechanism of action; c) determine whether it inhibits prolactin-binding in the lactating mammary gland, and in the mammary carcinomas induced by dimethylbenz(a)anthracene (DMBA) and N-nitrosomethylurea (NMU); d) study the effect of PBIF on the growth of DMBA and NMU-induced mammary carcinomas, which are known to be prolactin-dependent; e) determine whether PBIF is produced by these tumors; and f) observe whether it affects the binding of hormones other than prolactin.

There is evidence that NMU produces its carcinogenic effect by methylating the guanine of DNA in the O6 position. In the rat, hypothyroidism decreases the activity of the 'DNA repair enzyme' that removes O6-alkylguanine. We have produced moderate hypothyroidism in the rat prior to administration of a single i.v. dose of NMU, by administration of propylthiouracil (PTU), and continued the PTU for an additional 14 days. Surprisingly, 3-1/2 months later the incidence of mammary carcinoma was significantly decreased in the PTU-treated rats compared to controls. We propose to selectively examine the effect of moderate hypothyroidism and hyperthyroidism on the induction phase and on the growth phase of NMU-induced mammary carcinomas, and to investigate the molecular mechanism of any observed effects on induction.

105. STUDY ON GROWTH AND REGRESSION IN RAT MAMMARY TUMORS

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The overall objective of this proposal is to study the biological processes regulating the growth and regression of 7,12-dimethylbenz(a) anthracene (DMBA)-induced mammary tumors in rats. Most DMBA-induced mammary tumors are hormone-dependent and their growth or regression is regulated by the host's endocrine status. This proposal is based on the principle that any tissue growth is a result of a net gain of synthetic processes over tissue degradation. Thus, tissue synthesis and degradation play equally important roles in tumor growth. Understanding these processes could lead to the development of means to control malignant growth. The following subjects are the areas of our interest for the period proposed: (1) Differential and synergistic roles of estrogen and prolactin in protein and DNA synthesis in DMBA-induced mammary tumors. (2) Interrelationships between synthesis and degradative processes in growing and regressing tumors. (3) Whether or not tumor regression involves processes of active synthesis.

BIBLIOGRAPHIC REFERENCES: C. Lee, L. Oliver, E.L. Coe and R. Oyasu. (1979). Lactate dehydrogenase in normal mammary glands and 7,12-dimethylbenz(a)anthracene-induced mammary tumors in Sprague-Dawley rats. Journal of the National Cancer Institute 62:193-199.

106. HORMONAL REGULATION OF BREAST CANCER

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The overall objective of this proposal is to elucidate the mechanism by which hormones regulate growth and differentiated function in the normal mammary gland, and how these regulatory mechanisms have deviated in hormone-dependent mammary cancer. Specific emphasis is placed upon the mechanism by which prolactin regulates milk protein gene expression, as a model system for understanding peptide hormone action in general. In addition, the mechanism by which the steroid hormone, hydrocortisone, potentiates the action of prolactin is under investigation. Mammary gland organ culture employing a chemically-defined medium is being utilized to study the interaction of these hormones. Structural gene (mRNA-derived) DNA clones of the four rat milk protein genes are being utilized to study the synthesis, processing and turnover of the individual milk protein mRNAs. Distinct high molecular weight precursors for each of these mRNAs have been identified with the largest-sized transcript being greater than 5Kb. The organization of these genes is being studied using total DNA mapping and by screening a rat DNA library for the presence of these genes. Several positive plaques have been identified and the characterization of these genomic clones is in progress. The chromosomal localization of these genes is also being studied using somatic cell hybrids between CHO and mouse L cells. The mouse casein genes have been localized to a subset of mouse chromosomes and the homologous cloned mouse casein genes are now being used to perform fine mapping studies.

The expression of the milk protein genes in hormone-dependent, DMBA-induced rat mammary carcinomas was only 1-2.5% of that observed in the normal gland under the same hormonal environment. This loss of differentiated function appears to occur in the majority of the tumor cell population (greater than 95%). Using molecular hybridization and cell-free translation the poly(A) plus and poly(A) minus RNA populations in the tumors and normal gland have been compared. No unique tumor-specific sequences were identified and the primary differences appeared to be in the different abundancies of certain RNAs, especially the milk protein mRNAs.

BIBLIOGRAPHIC REFERENCES: Rosen, J.M. and Shields, D.: Post-translational modifications of the rat mammary gland caseins: in vitro synthesis, processing and segregation In: Testicular Development, Steinberger, A. (ed.), Raven Press, N.Y., p. 343, 1980. Matusik, R.J. and Rosen, J.M.: Prolactin regulation of casein gene expression: Possible mediators, Endocrinology, 106: 252-257, 1980.

107. FETAL EXPOSURE TO HORMONES AND MAMMARY CARCINOGENESIS

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This project will investigate the mechanisms of initiation, promotion and suppression of mammary tumors in the rat and will extend our analysis of the effects of prenatal exposure to diethylstilbestrol (DES) on mammary carcinogenesis.

Some of the DES-exposed female rats will be treated postnatally with a carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA). Conditions and drugs which modify the endocrine status of the DES-exposed animals before DMBA treatment will be employed to determine the effects of reproductive history or altered hormone levels on mammary tumor incidence.

The influence of prenatal exposure and postnatal treatment with estrogens on mammary tumor incidence will be compared in two different strains of rat. Data from these experiments will bear on the genetic aspects of susceptibility to tumor initiation.

Transplacental exposure to DES in humans is associated with teratogenic and carcinogenic changes in the vagina and cervix. Whether breast tissue was also affected is not yet known. Therefore, experiments in this proposal provide a model system where the potential risk for breast cancer among DES-exposed women can be studied.

108. CARCINOGEN-INDUCED CHANGES IN STEROID HORMONE ACTION

Rao, B. R., Washington University, School of Medicine, Dept. of Radiology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

We previously reported that 7,12-dimethylbenz(a)anthracene (DMBA) competes with progesterone for progesterone serum carrier protein(s) and target tissue receptors. In addition, we established that DMBA is a mild progestational agent. DMBA administration increases the uterine weight and induces endometrial glandular differentiation in estrogen primed rabbit. Additionally. DMBA treatment reduced uterine estrogen receptor level to below the detectable level on day 20 and there was a three-fold increase in progesterone receptor level. DMBA injected into mammary pads of rats and rabbits induced hyperplasia in stromal cells following 10 days of treatment and increased the progesterone receptor levels, while there was no change in estrogen receptor levels. Attempts to separate those hyperplastic cells from other mammary cells based on size and density using albumin gradient centrifugation was not successful. In order to detect whether estrogen receptors are present in those hyperplastic cells induced by DMBA, a specific fluorescent probe was developed by conjugating fluorescein to estrogen. We are now able to detect cells containing estrogen receptors using the fluorescein conjugated estrogen and will use this technique to detect cells containing estrogen receptors. We plan to use a similar fluorescent probe to detect progesterone receptors. The fluorescent probe technique may provide a means to detect those cells in which there is either an increase or decrease in estrogen and progesterone receptors.

109. CHEMICAL CARCINOGENESIS STUDIES WITH CULTIVATED HUMAN MAMMARY CELLS

Janiaud, P., Delaforge, M., Habart, M. H., Fondation Curie, Institut Curie, *11 rue Pierre et Marie Curie, 75231 Paris Cedex 5, France*

OBJECTIVE: To characterize the metabolic pathway present in human tissue (breast): Try to identify the regulation levels of monooxygenases and epoxide hydratase, and their relative level. Try to investigate some growth factors (from serum and lipids), try to determine the effects of several hormones. Obtain if possible some molecular markers (proteins elaborated by epithelial cells, such as glycoproteins (antibodies), and macromolecular secretion products. Set up an in vitro transformation system to score potential harmful molecules or associations of molecules.

APPROACH: Comparison of metabolic pathways from tissue homogenates and from cell cultures. Cultures enriched with epithelial cellular type are already possible. Evidence of epoxide diol metabolic pathway with several substrates such as safrole, DMBA, MCA has already been obtained. Relative level of epoxide hydrase as compared with monoxygenase production is comparatively weak, thus indicating a situation of relative molecular risk. Effects of several hormones on the metabolism level appear to be certain, and may regulate the levels of ultimate carcinogens produced by the mammary cell cultures.

Comparison with rat liver cell is now well developed, and allows direct study between mammary cell metabolism and liver cell metabolism. Cocultivation of mammary cells with rat liver cells is now possible, so that we could establish, in the same dish, a metabolic compartment and a target compartment.

PROGRESS: The culture technique appears to be reliable; several replicative cultures are already under study. Metabolic study with safrole is well developed, several metabolic patterns are under study for DMBA and MCA.

110. STEROID AND POLYCYCLIC AROMATIC HYDROCARBON MECHANISMS IN MAMMARY TUMORIGENESIS AND LEUKEMIA

Okey, A. B., University of Windsor, Dept. of Biology, *Windsor, Ontario*, N9B 3P4, Canada

OBJECTIVES: To determine the mechanisms by which steroids and polycyclic aromatic hydrocarbons interact in induction of mammary tumors; to determine the role of microsomal enzyme systems in activation and detoxification of aromatic hydrocarbons.

APPROACH: The effect of parent hydrocarbons and their metabolites on binding of estradiol to estrogen receptor in mammary cytosol is being assessed. The activity of aryl hydrocarbon hydroxylase in microsomes from mammary tissue during different states of susceptibility is being measured.

PROGRESS: Aryl hydrocarbon hydroxylase activity is detectable in the mammary tissue of Sprague-Dawley rats as young as 22 days of age. Basal enzyme activity increases about the time of puberty, then decreases before 50 days of age and remains low throughout ages up to 444 days. Pretreatment of rats with beta-naphthoflavone (BNF) induces a 6-70 fold increase in mammary AHH activity. Inducibility of AHH activity by BNF is greatest in older rats (which have a low sensitivity to tumor induction by polycyclic aromatic hydrocarbons). The inducibility of AHH activity by BNF rises dramatically in mammary tissue during late pregnancy and early lactation.

BIBLIOGRAPHIC REFERENCES: Fysh, J.M. and A.B. Okey, Biochem. Pharmacol. 27:2968-2972 (1978). Fysh, J.M. and A.B. Okey, Canad. J. Physiol. Pharmacol. 57:112-117 (1979).

111. METABOLISM OF PAH IN THE INDUCTION OF MAMMARY TUMORS

ElHawari, M., Midwest Research Inst., *425 Volker Blvd., P.O. Box 27-303, Kansas City, Missouri*, 64180, United States of America

Ascertain the role of metabolism in the induction of rat mammary tumors by certain polycyclic aromatic hydrocarbons (PAH) and to determine whether alternation in the disposition, metabolism, binding, and dna repair are related to the differences in susceptibility of certain strains, ages, and altered hormonal conditions. These objectives will be pursued through investigation of the distribution of the carcinogens at various levels in the host, their biotransformation, and their interaction with tissue macromolecules.

112. HORMONAL INHIBITION OF EXPERIMENTAL BREAST CARCINOGENESIS - MECHANISMS OF ACTION

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OBJECTIVE: To determine how progestational states occurring relatively early in life confer on the breast a partial resistance to cancer induction.

APPROACH: (1) Animals used - Random-bred Sprague-Dawley rats. (2) Pathological techniques - DMBA intragastric instillation and also direct dusting of mammary gland. (3) Histological techniques - Paraffin sections; mammary gland whole mounts; histochemical studies. (4) Biochemical techniques - Radioimmunoassays of plasma and pituitary PRL and GH; mammary epithelial cell isolation and fractionation; assays of hepatic and mammary microsomal enzymes, e.g., aryl hydrocarbon hydroxylase.

113. PITUITARY AND THYROID FUNCTION IN BREAST CANCER

Rose, D. P., Davis, T. E., Ruzicka, F. J., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology, *450 N. Randall Ave., Madison, Wisconsin*, 53706, United States of America

The objective of this project is to define the roles played by thyroid hormones and prolactin in the etiology and biology of breast cancer. This goal will be met by studies of nuclear triiodothyronine, cell membrane prolactin, and cytoplasmic estrogen receptors, and mitochondrial alpha-glycerophosphate dehydrogenase in experimental rat mammary carcinomas induced by N-nitrosomethylurea, and human breast cancer tissues. The influence of thyroid function and circulating prolactin levels on the receptor proteins and the thyroid hormone-inducible mitochondrial enzyme will be studied. Clinical investigations will be concerned with the influence of previous drug administration on tumor receptor levels, particular attention being paid to those known to alter plasma prolactin and estrogen levels.

BIBLIOGRAPHIC REFERENCES: Rose, D.P. and Davis, T.E. Plasma triiodothyronine concentrations in breast cancer. Cancer, 43:1434-1438, 1979. Rose, D.P., Pruitt, B., Stauber, P., Erturk, E. and Bryan, G.T. Influence of dosage schedule on the biological characteristics of N-nitrosomethylurea-induced rat mammary tumors. Cancer Res., 40: 235-239, 1979.

114. STUDY OF THE EARLY PHASES OF RAT MAMMARY CARCINOGENESIS BY ADRIAMYCIN AND DAUNOMYCIN AND THEIR POTENTIAL ONCOGENIC EFFECT IN MICE

Bucciarelli, E., Provvidenza, M., Universita degli Studi di Perugia, Istituto di Anatomia e Istologia Patologica, *Colle Obito, Box 327, 06100 Perugia Monteluce, Italy*

OBJECTIVES: Project I: To study the changes in the mammary gland and in the endocrine or endocrine-dependent organs of male or female Sprague-Dawley rats from the administration of Adriamycin (Ad) or Daunomycin (Dm) to the development of the mammary tumors (MTs). Project II: To test the oncogenic activity of Ad or Dm in mice. Project III: To study the effect on mammary carcinogenesis of sexual hormones in male and female Ad or Dm treated rats.

APPROACH: I) Male and female rats, intravenously treated at the age of 30-36 days with Ad (5mg/Kg), Dm (10mg/Kg) or NaCl (0.9%) have been killed at regular intervals from 10 to 100 days after treatment. The mammary glands are studied histologically and with the whole mount technique. The morphological and weight changes of the endocrine or endocrine-dependent organs are also being studied. II) Male and female BALB/c mice have been treated intravenously with 5mg/Kg of Ad, 10mg/Kg of Dm or with saline and killed at an average age of 451 plus or minus 8 days. III) Male rats will be castrated; castrated and transplanted with ovaries; or intact male rats will be transplanted with pituitaries from their litter mates. Female rats will be ovariectomised. Fifteen to twenty days following the operation the rats will be injected with Ad or Dm and observed for one year.

PROGRESS: I) A series of microscopic changes preceding the MTs appearance have been seen in the material so far examined, together with modifications in the endocrine organs. II) No significant difference in tumor incidence between treated and control mice has been observed. In treated animals, however, renal lesions were seen. III) Animals are now being treated.

115. NEUROENDOCRINE CONTROL OF LEUKEMOGENESIS

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OBJECTIVE: Define the hormonal environment prerequisite for the development and growth of carcinogen-induced leukemia in Long-Evans rats, determine the effect of drug-induced alterations of the activity of adrenergic, dopaminergic and serotoninergic pathways of the central nervous system on progression of carcinogen-induced leukemia in Long-Evans rats, demonstrate control of the growth of carcinogen-induced leukemia in Long-Evans rats by suppression of pituitary hormone secretion by administration of neuroendocrine influencing drugs.

APPROACH: Leukemia will be induced with chemical carcinogens by gastric incubation and selected groups will be altered in their hormonal status by ovariectomy, orchidectomy, hypophysectomy or hormone inoculations. Hemologic status of other groups will be altered by known active drugs. Other rats will have anterior pituitary secretion inhibited chemically. Pathologic assessments of leukomogenesis will be made under various hormonal pictures.

PROGRESS: The objectives of this study were: 1. To define the hormonal environment prerequisite for the development and growth of carcinogen-induced leukemia in Long-Evans rats. 2. To determine the effect of drug-induced alterations of the activity of adrenergic, dopaminergic and serotoninergic pathways of the central nervous system on progression of carcinogen-induced leukemia in Long-Evans rats. Results: Long-Evans rats were: ovariectomized, orchiectomized, treated with 17 Beta-estradiol, testosterone, thyroxine or thiouracil. None of these treatments significantly influenced the development and/or growth of TMBA (or DMBA) induced murine leukemia. Long-Evans rats were treated with: L-dopa (dopamine agonist), methyl dopa (dopamine antagonist), PCPA (serotonin antagonist), chlorpromazine (dopamine antagonist), Alpha MPT (adrenergic antagonist), reserpine (depletion of catecholamines), clonidine (norepinephrine agonist), piribedil (dopamine agonist) or naloxone (opiate antagonist). None of these treatments significantly influenced the development and/or growth of TMBA (or DMBA) induced murine leukemia. These results provide evidence indicating that the polycyclic hydrocarbon induced leukemia in the Long-Evans rat is not responsive to alterations in activity of the neuroendocrine system.

116. INFLUENCE OF ENVIRONMENTAL POLLUTANTS ON ENDOCRINE ORGANS - MECHANISMS FOR THE DEVELOPMENT OF HORMONE ABNORMALITIES, NECROSIS AND CANCER

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OBJECTIVE: The objective is to clarify the mechanism for the development of necrosis and cancer in endocrine (steroidogenic) organs and its relationships to existing steroid metabolizing systems.

APPROACH: The metabolism and regulation of mainly polycyclic hydrocarbons are studied in adrenals, ovaries and testes from the rat, cow, dog and human, as well as in cultured cells from these organs. Effects of steroids and peptide (pituitary) hormones on this metabolism are investigated in vivo and in vitro with HPLC techniques.

PROGRESS: 7,12-Dimethylbenz(alpha)anthracene (DMBA) and benz(alpha)pyrene (BP) are metabolized by cytochrome P-450-dependent hydroxylase(s) in microsomes from rat adrenals. This metabolism generates mainly phenols and diols. Metabolism is associated with covalent binding of metabolites to soluble and microsomal proteins, and is inhibited by various compounds including estradiol. The possibility that DMBA metabolism involves the estradiol-synthesizing system is considered.

The direct toxic effects of DMBA appear to be related to binding of not yet identified reactive products to essential protein, e.g., glutathione-S-transferases, and a rather specific effect of some metabolites on mitochondrial function. This latter effect seems to be the major contribution to the toxicity and may involve inhibition of mitochondrial protein synthesis.

117. HISTOPATHOLOGY AND CARCINOGENESIS OF HUMAN PROSTATE

Trump, B. F., Heatfield, B. M., Kahng, M. W., Purnell, D. M., Sato, T., Universities Associated for Research & Educ. in Pathol. Inc., Dept. of Pathology, *9650 Rockville Pike, Bethesda, Marylanc.* 20014, United States of America

The overall objectives of the proposed research are: 1) to characterize alterations in human prostate carcinoma at the subcellular level utilizing electron microscopy (TEM, SEM) and immunocytochemistry to further improve prognostication based on Mostofi's histopathologic grading system, 2) to study differentiation of untreated, and tumor potential of carcinogen-treated, cultured explants via xeno-transplantation into nude (athymic) mice, 3) to examine modulation of carcinogenesis in vitro by vitamin A analogs, and steroid hormones (testosterone, dihydrotestosterone, and estradiol), 4) to investigate DNA binding and production of metabolites by radiolabeled dimethyl benzanthracene and effects of steroid hormones on aryl hydrocarbon hydroxylase induction, and 5) to determine the distributional patterns of hormone binding sites in vitro and in retrospective, histopathologic material using radio-autographic detection of tritium-labeled steroids.

BIBLIOGRAPHIC REFERENCES: Trump, B.F., Heatfield, B.M., Phelps, P.C., Sanefuji, H., and Shamsuddin, A.K.: Cell surface changes in preneoplastic and neoplastic epithelium. SEM III 1979, SEM Inc., AMF O'Hare, Illinois, 60666, pp. 43-60.

118. THE EFFECT OF HEPATOCARCINOGENS AND ORAL CONTRACEPTIVE FORMULATIONS ON RNA TRANSPORT FROM ISOLATED RAT LIVER NUCLEI (ABBREV)

Blunck, J. M., Deakin University, Division of Biological & Health Sciences, *Geelong, Victoria 3217, Australia*

OBJECTIVE: To determine whether alterations in liver RNA transport induced by feeding hepatocarcinogens to rats are modified by concurrent administration of some oral contraceptive formulations.

APPROACH: Groups of 6 Sprague-Dawley rats of both sexes will be pair-fed either a control diet or diets containing the following carcinogens in doses that are capable of inducing tumors during the lifespan of the animals: 3'-methyl-4-

dimethylaminoazobenzene (3'MeDAB), 2-acetylaminofluorene (AAF), ethionine and aflatoxin B1. Further groups of rats will be simultaneously pair-fed for 4 days on each of these carcinogen-containing diets in combination with mestranol, norethynodrel-mestranol 25:1 or 17 beta-oestradiol (dose, 20 x the human dose on a weight basis). Shortly before sacrifice, liver nuclear RNA will be labelled in vivo by i.p. administration of 14C-orotic acid. The rats will then be killed, the livers removed, and the labelled nuclei isolated by ultracentrifugation and incubated in a medium containing ATP and cytosol proteins in order to assay RNA release which is determined by measuring acid precipitable radioactivity in the medium after assay.

119. EXPERIMENTAL PANCREATIC CARCINOMA - MODULATING EFFECTS OF GASTROINTESTINAL POLYPEPTIDE HORMONES AND ZINC

Barrowman, J. A., Pfeiffer, C. J., Memorial University of Newfoundland, School of Medicine, *Health Science Complex, St. Johns, Newfoundland,* A1C 5S7, Canada

OBJECTIVE: The study aims to investigate the possible co-carcinogenic action of chronic administration of the C-terminal octapeptide analogue of cholecystokinin (CCK-OP) on the pancreas of Syrian hamsters which are currently receiving the powerful pancreatic carcinogen, N-nitrosobis (2-oxopropyl) amine (BOP). The other component of the study is to examine the possible co-carcinogenicity of zinc deficiency in the same experimental model.

APPROACH: The initial experiments are designed: 1) To establish in the Syrian hamster that chronic injections of CCK-OP are trophic to the pancreas, as has been shown in other species. 2) To determine whether concurrent CCK-OP injections enhance the incidence of pancreatic adenocarcinomata in hamsters receiving BOP injections. 3) To examine BOP-induced carcinogens in the presence of normal dietary zinc intake and under conditions of zinc deprivation. 4) To examine the effects of combined zinc deficiency and CCK-OP administration on BOP-induced carcinogens.

All the studies of carcinogenesis will involve 13-week periods of study. In part 1, the trophic actions of CCK-OP on the pancreas will be studied over a 10-day period and a 13-week period. Suitable control animals will be included in all the studies. Pancreatic wet and dry weights and protein, collagen, DNA, RNA and amylase content will be measured together with light and electron microscopic studies of cell size and morphology. Tumor incidence and histopathology will be assessed by standard techniques.

120. ENDOCRINE FACTORS IN THE DEVELOPMENT OF PLASMACYTOMA

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A number of endocrine factors have been found to influence the development of plasmacytoma in the pristane-injected BALB/c mice. The discovery of two non-endocrine factors, bacterial lipopolysaccharide and pristane-induced peritoneal factors, has led to the development of an hypothesis integrating the profound effects of certain endocrine agents on the development of this tumor. Bacterial lipopolysaccharide administration increases the development of tumors in pristane-injected mice at some early step which produces nascent tumors, which cannot readily be transplanted to syngeneic mice. Intraperitoneal administration of pristane produces a factor in peritoneal fluid within three days which allows ready transplantation of primary tumors to appropriate hosts. This material contains no pristane. It is proposed to relate the endocrine factors that either accelerate or prevent tumorigenesis to the increase in peritoneal cells and bacterial lipopolysaccharide produced by intraperitoneal pristane, to a transformation step, to nascent tumor cells, or to a maturation step induced by our pristane-induced peritoneal factor.

121. ACTIONS OF CHEMICAL CARCINOGENS ON CULTURED CELLS

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Dehydroepiandrosterone (DHEA) is a major adrenal secretory product in men and women, yet it has no known biological function. On the basis of a 10-year prospective study involving over 5000 apparently healthy women, Bulbrook et al. concluded that women with subnormal excretory rates of androsterone and etiocholanolone (metabolites of DHEA) experience an increased risk of breast cancer. It is well documented that DHEA is a potent non-competitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PD). Yen et al. found that long-term treatment of mice with DHEA reduces weight gain without suppressing appetite, presumably by inhibiting lipogenesis by reducing NADPH levels as a result of inhibition of G6PD. We have found that long-term treatment of C3H-Avy/A (obese) and C3H-A/A (non-obese) mice with DHEA, in addition to reducing weight gain without suppressing appetite, markedly inhibits the development of spontaneous breast cancer and delays the rate of aging. We have since observed that DHEA is a potent anti-tumor promoter, and antagonizes the epidermal cell hyperplasia and enhancement in the rate of DNA synthesis when phorbol esters are applied to mouse skin.

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122. SYNTHETIC STEROIDS AND HEPATOCARCINOGENESIS

Vesselinovitch, S. D., University of Chicago, Pritzker School of Medicine, Dept. of Radiology, *5801 S. Ellis Ave., Chicago, Illinois,* 60637, United States of America

There is enough evidence that sex hormones may influence promoting phase of hepatocarcinogenesis apart from their effect upon initiation which operates through enzyme system activating chemical carcinogens. Clinical and experimental data show that males develop liver tumors more readily than females. Also, change in hormonal environment triggered by gonadectomy or administration of sex hormones following initiation modifies incidence of liver tumors, indicating strongly its modulating effect upon promotion of neoplastic process. Recent clinical observations suggest that use of oral contraceptives and anabolic steroids may be causally related to observed increase in the incidences of focal nodular lesions and even hepatocellular carcinomas in individuals using such hormones for long periods. The hypothesis that synthetic hormones may also achieve their effect by influencing promotion could be tested and defined in a system developed in our laboratory, in which single low dose levels of diethylnitrosamine (DEN) (less than or equal to 0.15 microgram/g body weight) were shown to be effective in initiating hepatocarcinogensis in C57BLxC3H F1 mice. Slightly higher, but still low, dose levels (0.6 to 5.0 micrograms/g body weight) were able to induce nodular liver lesions, ranging from hyperplastic to adenomatous and malignant cell populations, their incidence depending upon the dose of carcinogen used and the length of observation period. In the proposed study, promoting effect of norethynodrel, mestranol, methyltestosterone, and oxymetholone will be tested in groups of mice conditioned by subeffective (initiating) and marginally carcinogenic (inducing) single dose treatments with DEN by assessing type and incidence of developing lesions. Possible weak carcinogenic activity of the above hormones will be concurrently assessed. Phenobarbitone (0.05%) will be used as positive promoter.

123. ENDOCRINE FACTORS IN TUMOR PROMOTION

Nawata, H., Poirier, L. A., Yamamoto, R. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nutrition & Metabolism Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The effects of hormones on the induction of ornithine decarboxylase (ODC), S-adenoxylmethionine decarboxylase (SAMDC), and polyamine levels in vivo are being studied. Previous studies have shown that ODC induction may be a marker for tumor promoters. Hormones often increase the biological activities of complete carcinogens. ODC is used to study the degree of biochemical similarity between hormones and promoters. The present studies indicate that estradiol induces

renal ODC and SAMDC in both the rat and hamster. The induction of renal ODC in the rat by estrogens is proportional to their binding in vitro to cytoplasmic estradiol binding sites. ODC levels in the ovary and uterus of female rats are inversely correlated with each other. Estrogens as potential promoters will be tested in two-stage initiation-promotion carcinogenesis studies.

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124. BIOLOGY OF MAMMARY NEOPLASIA

Nandi, S., Bern, H. A., Blair, P. B., DeOme, K. B., Pitelka, D. R., University of California, Berkeley Campus, School of Letters & Science, Dept. of Zoology, *Berkeley, California,* 94720, United States of America

The summary for this project is under entry no. 100.

125. ENDOCRINOLOGY OF EXPERIMENTAL LEUKEMIA

Huggins, C. B., University of Chicago, Pritzker School of Medicine, Dept. of Surgery, *5801 S. Ellis Ave., Chicago, Illinois,* 60637, United States of America

A large group of rats receives a carcinogenic dose of chemicals which selectively induces mammary cancer and leukemia in 100% of the animals. At a later date, hormones are given or endocrine surgery is performed which will cure or mitigate the severity of the leukemias and mammary carcinomas.

BIBLIOGRAPHIC REFERENCES: Huggins, C. B. 1979. Experimental Leukemia and Mammary Cancer: Induction, Prevention, Cure. The University of Chicago Press, Chicago and London, pp. 221.

IV. MODIFICATION OF CHEMICAL CARCINOGENESIS BY DIETARY AND DIGESTIVE FACTORS

A. ENHANCEMENT BY DIETARY COMPONENTS

126. CHOLINE DEFICIENCY, OVAL CELLS AND HEPATOCARCINOGENESIS

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The research proposed in this application is designed to study two aspects of chemical hepatocarcinogenesis: 1) the influence of a dietary deficiency of a single nutritive factor, choline, on tumor induction; and, 2) the nature and functional properties of so-called oval cells. Feeding a diet devoid of choline to rats has been shown to promote the induction of preneoplastic lesions, as well as of hepatocellular carcinomas, by several known chemical carcinogens. Studies are proposed aimed at investigating possible mechanism(s) whereby the diet acts as a promoter of liver carcinogenesis. Mechanisms to be considered are: 1) stimulation of hepatocyte replication; 2) diet and carcinogen-induced changes in the properties of hepatocyte membranes; and, 3) transmethylation reactions involving methylation of hepatocyte macromolecules. The influence of the diet on initiation of liver carcinogenesis will also be investigated. In recent years, a renewed interest has developed about the possibility that bile duct(ular) cells, beside hepatocytes, may also be target cells of chemical hepatocarcinogens, and progenitors, in certain instances, of hepatocellular carcinomas as well as of cholangiocarcinomas. Proliferation of bile duct(ular) cells, with emergence of so-called oval cells, is a common, early event occurring in the liver of rats exposed to chemial carcinogens. Studies are proposed aimed at achieving the isolation of homogeneous preparations of oval cells from the liver of carcinogen-treated rats; at investigating some of the metabolic and biologic properties of these cells; and, at probing whether these cells do indeed play any role in the genesis of epithelial liver tumors.

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127. METABOLISM AND CO-CARCINOGENICITY OF CYCLOPROPENES

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Cyclopropenoid fatty acids (CPFA) which occur in certain foodstuffs such as 'Cot-N-Nuts,' cottonseed oil and flour; kapok oil and okra are known to have toxic effects when consumed by animals. These unique compounds are known to impair reproduction in rats and chickens, alter the fatty acid composition in the body lipids of several species, and cause characteristic alterations in trout and rabbit liver parenchymal cells. In addition, our studies have demonstrated that CPFA act as powerful cocarcinogens with aflatoxin B1 and as carcinogens in the induction of liver cancer in rainbow trout.

This research shall have as the primary goals an explanation for the cocarcinogenic and carcinogenic activity of CPFA, and the expansion of our knowledge of their toxic effects in trout and other animals. To attain this goal the effect of CPFA on the microsomal mixed function oxidase system and on the activation and detoxication of aflatoxin B1 will be determined and correlated with induction of tumors. The effect of CPFA on microsomal membrane lipid composition and structure will be investigated to determine if CPFA have an indirect effect on enzyme activity by influencing the membrane lipid environment of the enzyme. The structural specificity required for cyclopropene activity will be investigated by measuring biological activity of malvalate, the predominant CPFA in foodstuffs, and hydrocarbon homologous analogs of CPFA.

128. MODULATION OF PANCREATIC CARCINOGENESIS BY DIET

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Preliminary data indicate that high dietary levels (20% by weight) of unsaturated fat act to enhance the incidence, mean number of tumors per pancreas, and the severity of the pancreatic tumors in azaserine-treated, male Wistar/Lewis rats. Azaserine is a known pancreatic carcinogen for this rat. Comparable levels of saturated dietary fats do not enhance azaserine induced pancreatic tumors. The enhancement appears to be true promotion as this effect is demonstrated to occur when the rats are initiated with azaserine and, during the post-initiation phase (when no further carcinogen is given), are fed the high levels of dietary fat.

The objectives of these studies are to generalize the above very specific observation and in doing so develop an understanding of the biochemical mechanism(s) of dietary promotion of pancreatic carcinogenesis. The proposed studies are: (1) Demonstrate that high dietary levels of unsaturated fat will promote pancreatic carcinogenesis in the established hamster model. (2) Develop short-term (ca 4 month) animal models for dietary promotion to circumvent the present expensive, time-consuming 1 plus year rat model. (3) Apply some of the biochemical parameters thought to be indicative of promotion in the mouse skin system and the rat liver system to dietary promotion of pancreatic cancer. (4) Investigate, hopefully by a short-term model, unsaturated fat promotion of other pancreatic carcinogens, other possible promoting agents in initiated pancreas, and dietary promotion in the opposite sexes of the established models (i.e., female rat and male hamster).

129. FREE RADICALS IN NITROSO-UNSATURATED LIPID REACTIONS

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The summary for this project is under entry no. 70.

130. DIETARY FAT AND MAMMARY CARCINOGENESIS

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This research project will conclusively demonstrate the effects of a high fat diet on the induction of mammary cancers in 3 strains of rats. The possible co-carcinogenic effect of a high fat diet in mammary cancer induction by a chemical carcinogen will be defined. The possibility that high dietary fat alters the susceptibility of the mammary gland to carcinogenesis as related to the age of the host, the rate of mammary cell proliferation and hormone biosynthesis will be investigated by studying whether a fat diet may increase the incidence of mammary cancer in post-pubertal rats; by examining whether a high fat diet accelerates the rate of DNA synthesis and cell proliferation; and by measuring the biosynthesis of steroid and polypeptide hormones. The possible relationship between increased hormone production and increased rate of mammary cell proliferation in rats ingesting a high fat diet may be delineated. A comparison of the efficacy of polyunsaturated and unsaturated fat in their co-carcinogenic effect may lead to the identification of the type of fatty acid in the high-fat diet causing enhancement of mammary tumorigenesis. Or the possibility that the amount of dietary fat consumed, rather than the specific type of dietary fat, may play a critical role in influencing the induction of mammary carcinogenesis may be ascertained.

131. EFFECTS OF ORAL ADMINISTRATION OF HIGH DOSES OF SALT ON MNNG-INDUCED GASTRIC CANCER IN RATS

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OBJECTIVE: Highly-concentrated salt intake is assumed to be a promotor of human gastric cancer. This hypothesis is being tested by animal experimentation.

APPROACH: Preliminary experiments using half-saturated doses of salt and low doses (20 microgram/l) of MNNG in Wistar rats provided supporting data for this hypothesis. Experiments now underway involve more large-scale planning.

PROGRESS: Evidence indicated that oral administration of low-doses of MNNG alone couldn't induce any kinds of tumors in rat glandular stomach within a certain period, while addition of high-dose salt intake during MNNG administration promoted a higher incidence of gastric tumors.

132. ETHANOL AS PROMOTER IN AFLATOXIN CARCINOGENESIS

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This is part of a broader project. A summary of this subproject is not available.

B. INHIBITION BY DIETARY COMPONENTS

133. EFFECT OF SELENIUM ON TUMOR GROWTH

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OBJECTIVE: Determine the involvement of the dietary nutrient, selenium, on the growth and development of neoplastic tissue. Determine which form(s) and concentration(s) of selenium is most deterimental to tumor tissue. These experiments will attempt to find a common mechanism of action of selenium on three different model systems for study of carcinogenesis.

APPROACH: These studies will determine the effect of dietary selenium supplementation on the growth of transplantable Ehrlich Ascites Tumor cells, dimethlhydrazine induced colonic cancer and the incidence of spontaneous leukemia in inbred mice. Studies will also compare the route of administration of selenium on the growth of transplantable tumor cells. The optimum quantity and form of selenium needed to retard tumor growth will be examined. The mitotic index of tumor cells will be used as an indicator of the effect of selenium on cellular division in both ascitic and solid Ehrlich tumor cells. The effect of selenium supplementation upon the key regulatory substrates and enzymes associated with cellular growth will also be examined.

PROGRESS: Numerous studies have been conducted in our laboratory which show that selenium supplementation can retard the growth of the transplantable Ehrlich ascites tumor cell, the L1210 leukemic cell and the Lewis lung carcinoma. Experiments conducted in the Ehrlich ascites tumor cells examined the influence of dose and form of selenium administered on the growth of this cell line. All forms were found to be completely effective in inhibiting the growth of this tumor cell line when administered to mice at a concentration of 2 Mug/g body weight. Reducing the concentration of selenium to 0.25 Mug/g body weight revealed that the inorganic forms of selenium were more efficacious in retarding tumor growth than the organic forms. Under the studies, seleno-cystine and seleno-methionine when injected into mice inoculated with Ehrlich ascites tumor cells were not as efficacious as sodium selenite, sodium selenate and selenodioxide. Similar studies have been conducted in mice inoculated with L1210 tumor cells. These studies revealed that sodium selenite and seleno-dioxide were the most efficacious in retarding tumor growth. Again in this tumor cell all seleno compounds examined resulted in tumor cell death in vitro as indicated by Trypan blue uptake. (Text Abridged.)

134. SELENIUM SUPPLEMENT AND DIETARY FAT IN BREAST CANCER

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The summary for this project is under entry no. 67.

135. INHIBITION OF CARCINOGENESIS BY SELENIUM

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The overall objective of the research project is the attainment of a better understanding of the molecular mechanisms underlying the protective effect of selenium against chemical carcinogenesis. Initial studies are being conducted to quantitate the effect of dietary selenium (0.5 ppm) on the incidence of hepatomas in rats fed 2-fluorenylacetamide (FAA). Subsequent experiments will focus on the possible relationship between the antiperoxidase (GPO). Specifically, an attempt will be made to correlate the activity of GPO in the livers of selenium-deficient and selenium-supplemented rats with hepatic malondialdehyde concentrations at various intervals after the addition of FAA to their diets.

Other studies will be concerned with the effect of selenium on the metabolism of FAA both in vivo and in vitro. Recently developed high pressure liquid chromatography techniques will be used to separate and quantitate metabolites of FAA excreted in the urine. By similar means the role of dietary selenium in the metabolism of FAA by hepatic microsomes in vitro will be quantitated.

Finally, the effect of selenium on the binding of FAA to hepatic DNA is being investigated. The effect of selenium on the amount of carcinogen initially bound to DNA as well as its effect on the retention of DNA-bound FAA at later intervals will be studied. In conjunction with the studies described above, these investigations should provide new insights into the ability of selenium to protect the liver against potential changes in gene function induced by FAA.

136. NUTRITION AND MAMMARY CARCINOGENESIS

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Investigations are proposed to study the effect of organic and inorganic forms of dietray selenium on the initiation, promotion and progression stages of 7, 12 dimethylbenz(a)anthracene (DMBA) and 1-methyl-1-nitrosourea (MNU) induced mammary carcinogenesis in the rat. The influence of selenium on the metabolism of DMBA will be studied

both in vivo and in mammary gland organ cultures in an attempt to evaluate the mechanism by which tumor initiation is inhibited. The role of pharmocological levels of selenium in blocking macromolecular synthesis will also be investigated. Other work will be undertaken to determine whether selenium kills established tumor cells. Life time studies of the inhibition of mammary carcinogenesis by selenium will be initiated.

137. BIOLOGY OF MAMMARY PRENEOPLASIAS

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The summary for this project is under entry no. 69.

138. CHLORINATED WATER AND MEMBRANE FUNCTIONS AND NEOPLASIA

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The summary for this project is under entry no. 11.

139. EFFECTS OF STEROLS AND BILE ACIDS ON COLON CANCER

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The overall objective of our research program is to delineate the role of sterols and bile acids in the etiology of large bowel cancer in animals and in man. Epidemiological studies have suggested that the major determinants in the development of colon cancer are environmental factors. Evaluation of the dietary habits of populations with different risks for colon cancer has shown that high risk groups consume diets with large portions of fat and beef whereas low risk populations typically eat food low in such components and rich in vegetable products. In view of the low incidence of colon cancer in populations consuming a diet which included large amounts of sterols of plant origin, we sought to determine whether these compounds retarded tumor formation in a rat model of colon cancer. Male Fischer rats were given 8 mg of the carcinogen N-methyl-N-nitrosourea (MNU) in 4 intracolonic instillations (over a 2-week period). Simultaneously, a 0.2% beta-sitosterol supplemented diet was fed. Analyses of fecal steroids using TLC, GLC and GLC-MS were sequentially carried out in each treatment group. At 28 weeks the incidence of colonic tumors was determined by pathological and histological examination. There were no adverse effects of beta-sitosterol feeding at the 0.2% level and no deaths occurred during the 28-week experiment. The fecal concentrations of bile acids and cholesterol (including bacterial metabolites) were similar in the experimental and control groups. The fecal concentration of plant sterols in the beta-sitosterol-fed rats was elevated approximately 7-fold compared to rats given control chow (7.6 mg/g dry feces vs. 1.2 mg/g). In rats given control chow plus MNU there were 22 out of 45 animals with tumors (49%) and 1.1 tumors/animal. In the rats given beta-sitosterol supplemented chow plus MNU, 15 out of 44 animals had tumors (33%) and there were 0.36 tumors/animal. Thus, beta-sitosterol reduced the number of tumors (adenomas, carcinomas in situ, and invasive carcinomas) from 50 to 16. These results lend credence to the possibility that plant sterols may have a tumor-retarding effect.

140. STUDIES OF NATURAL INHIBITORS OF CHEMICAL CARCINOGENS

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OBJECTIVES: Identify naturally occurring non-toxic materials suitable for addition to normal diets in order to inhibit the development of cancer of the large bowel and breast cancer. Specifically, assess the effects of a variety of dietary fibers on chemically induced colon and breast carcinogenesis in an animal model.

C. MODIFICATION BY DIETARY AND DIGESTIVE FACTORS

141. DIETARY ARGININE AND TUMOR GROWTH AND DEVELOPMENT

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This research proposal is designed to determine the role of dietary arginine in the etiology of oncogenic and carcinogenic growth and development. The primary experimental animal model used in this research will be the mammary tumor induced by 7, 12-dimethylbenzanthracene (DMBA) in the rat. Products and enzymes of intermediary metabolism will be examined in all tumor tissue and animal excreta to determine the biological effects of dietary supplementation with arginine. Diets will be varied in carefully controlled and predetermined ways to assess the involvement of arginine in tumorigenesis. The effect of arginine precursors and metabolities, such as ornithine, citrulline, urea, and putrescine as dietary supplements on tumor growth and incidence, will also be examined. The percentage of animals with tumors will be examined in all experiments. The effect of arginine or its metabolites on the binding of DMBA to mammary tissue will be determined. Furthermore, the influence of arginine on the tissue and urinary metabolites of DMBA will be examined by high pressure liquid chromatography. Some studies will examine the influence of arginine supplementation on the Salmonella typhimurium mutation test. These studies will also examine the influence of arginine supplementation as a therapeutic agent in the treatment of transplantable tumors. These studies will be conducted in vivo and in vitro with Ehrlich Ascites tumor cells. The effect of arginine supplementation on cell cycle kinetics and macronutrient biosynthesis will be examined. These studies will add valuable information on the therapeutic values of supplemental arginine. The overall objective of this research is to supply nutritional information in the relationship of diet to the growth and development of oncogenic and carcinogenic tissue. Additionally, these studies will attempt to find a common link between model systems used in cancer research in regard to their association with dietary arginine.

142. PROTEIN EFFECTS OF AFLATOXIN CARCINOGENESIS IN TROUT

Sinnhuber, R. O., Hendricks, J. D., Nixon, J. E., Lee, J. S., Bailey, G. S., Casteel, J. L., Oregon State Higher Education System, Oregon State University, School of Agriculture, Dept. of Food Science & Technology, *35th & Jefferson Sts., Corvallis, Oregon*, 97331, United States of America

The rainbow trout (Salmo gairdneri) will be used to study the effects of dietary protein on hepatocellular carcinoma (hepatoma) induction and development. Aflatoxin B1 (AFB1), a useful secondary carcinogen giving consistent results in laboratory animals, will be utilized to induce neoplasia in the fish. Rainbow trout are the most sensitive known experimental animals as regards carcinogenic properties of AFB1 and are similar to humans and nonhuman primates in their in vitro metabolic conversion of AFB1. This model provides a convenient test system in which to determine the effects of diet on tumor induction and growth. In addition, exposure of the trout eggs to AFB1 for a brief period of time will yield a population of latent cancer-bearing animals in which dietary parameters can be evaluated.

The effects of dietary protein on AFB1 carcinogenesis and biochemical mechanisms through which these effects are exerted will be investigated by combining in vivo studies of tumor production in trout along with in vitro studies on the enzymatic activation and detoxification of AFB1, identification and quantification of AFB1 metabolities, and conversion of AFB1 to active bacterial mutagens.

BIBLIOGRAPHIC REFERENCES: Dietary Casein Levels and Aflatoxin B1 Metabolism in Rainbow Trout (Salmo gairdneri). W. T. Stott and R. O. Sinnhuber. ACS Symposium Series, Book 99, Chapter 23. Metabolism and Deposition of Pesticides and Other Chemicals in Aquatic Organisms. pp. 389-400 (1979). High Dietary Protein Promotes Embryo-Induced Aflatoxin Carcinogenesis in Rainbow Trout. J. D. Hendricks, J.

E. Nixon, P. M. Loveland, J. L. Casteel and R. O. Sinnhuber. Federation Proceedings 40:000 (1981).

143. DIETARY PROTEIN-FAT INTERACTIONS IN DMBA MAMMARY CANCER

Visek, W. J., Truex, C. R., Clinton, S. K., University of Illinois, Urbana Champaign Campus, School of Basic Medical Sciences, *Medical Sciences Bldg., Urbana, Illinois*, 61801, United States of America

The objective will be to determine how the quantities of two major dietary nutrients, protein and lipid, interact to influence 7,12-dimethylbenz(a)anthracene (DMBA) mammary carcinogenesis in rats. Different concentrations of dietary protein and lipid will be fed in casein-containing diets with the following micrograms of protein per kilocalorie of dietary energy: 20, 30 and 80. Lipid will be supplied as corn oil at 13, 26 and 54 micrograms per kilocalorie of dietary energy at each of three dietary levels of protein. Minerals, vitamins and fiber will be supplied at a constant ratio per kilocalorie of dietary energy for all dietary treatments. Experiments are designed to study dietary influences on the initiation and promotion phases of carcinogenesis. The absorption, tissue distribution and excretion of DMBA and its metabolites will be determined. Binding of DMBA metabolites to RNA, DNA, and protein of mammary tissue will be studied. Metabolites in urine and feces will be identified and quantitated. Liver aryl hydrocarbon hydroxylase and related mixed function oxidase components will be assayed to determine how the dietary variables influence their activity. All tumors will be studied histologically.

BIBLIOGRAPHIC REFERENCES: Clinton, S.K., C.R. Truex, P.B. Imrey and W.J. Visek. Dietary protein and mixed function oxidase activity. In Microsomes, Drug Oxidations, and Chemical Carcinogenesis. (M.J. Coon, ed.), Academic Press, 1980 (in press). Clinton, S.K., R.J. Destress, D.B. Anderson, C.R. Truex, P.B. Imrey and W.J. Visek. 1,2-dimethylhydrazine-induced intestinal cancer in rats fed beef or soybean protein. Nutr. Rpts. Intnl. 20:335-342, 1979.

144. RELATIONSHIP OF DIET AND INTESTINAL CANCER

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OBJECTIVE: Determine if bacterial metabolites of bile acids, cholesterol or amino acids are mutagenic or comutagenic. Determine if protein, fat or fiber content of the diet affects the frequency and type of chemically induced tumors in mice.

APPROACH: Mutagenic and comutagenic properties of bacterial metabolites of bile acids, cholesterol and amino acids will be evaluated with fetal rat intestinal cell cultures. The frequency of cell transformation from ouabain sensitive to ouabain resistant cells will be the criteria for mutagenesis. Colon tumors will be produced in CF1 mice by injections of 1,2-dimethylhydrazine. The frequency and types of tumors will be correlated with the quantity of protein, fat and fiber in the diet. Also, colon contents of the mice will be assayed for type and quantity of steroids.

PROGRESS: Bile acids and other gut metabolites are thought to play a role in the etiology of colon cancer. The role of various gut metabolites in either initiation or promotion of carcinogenesis was tested on mouse skin. Deoxycholic acid, lithocholic acid, and cholestanone enhanced, and 3,12-diketocholanic acid inhibited dimethylbenz(a)anthracene initiated and croton oil promoted tumorigenesis. Cholic acid, chenodeoxycholic acid, cholesterol, cholestanol, skatole, indole, and p-cresol had no effect on tumorigenesis. None of the gut metabolites acted as initiators or promoters of carcinogenesis; however, several steroids appeared to have a 'permissive' action in croton oil promotion. Diet may influence colon carcinogenesis through certain gut metabolites, but diet is not likely to cause colon cancer. Cultured colon cells from rats were used to study the metabolism of ingested environmental contaminants. Cells metabolized benzo(a)pyrene and bound tritiated benzo(a)pyrene to cellular macromolecules. Culturing cells in the presence of lithocholic or deoxycholic acids increased benzo(a)pyrene metabolism. Also, cells metabolized 1,2-dimethylhydrazine, a carcinogen which produces neoplasms predominantly in colon epithelial cells. Deoxycholic and lithocholic acids had no effect on 1,2-dimethylhydrazine metabolism.

145. NUTRITION AND EXPERIMENTALLY INDUCED LUNG CANCER IN HAMSTERS AND RATS

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Epidemiological data suggest that nutritional habits may be of importance for the occurrence of various types of cancer, such as cancer of the breast, prostate and colon. Tobacco smoking is an important causative factor for lung cancer. However, some epidemiological findings and animal experiments suggest that, in addition to smoking, dietary factors might influence lung cancer incidence. These factors include fat and caloric intake, vitamin A level of the diet and intake of antioxidants.

The aim of the present project is to investigate the importance of nutrition in the prevention of chemically induced lung cancer in laboratory animals. Lung tumors are induced in Syrian golden hamsters by intratracheal intubation of benzo(a)pyrene and ferric oxide. Groups of 40 hamsters of each sex are fed various diets during the whole experimental period (1.5 year). Tested are a diet high in saturated fat ad libitum, a diet high in unsaturated fat ad libitum, a control diet (low fat) ad libitum and the same diets restricted (80% of the energy taken by ad libitum fed animals). Furthermore diets with various levels of vitamin A are tested. The incidence of lung tumors and metaplastic changes and the pattern and incidence of metastases in the various dietary groups are recorded.

Similar experiments are performed in rats. In these studies attention will also be paid to the role of antioxidants in the diet (vitamin E and selenium). Moreover, the role of the immunocompetence of animals on various diets, a factor that might be of importance for the growth and spread of tumors, is investigated.

If dietary factors exert a modifying effect on chemically induced lung tumors in animals the nutrition might also have preventive and perhaps therapeutic possibilities in humans. The studies are in progress.

146. DIET, FLORA AND COLON TUMORIGENESIS

Broitman, S. A., Gottlieb, L. S., Vitale, J. J., Panda, S. K., Boston University, School of Medicine, Dept. of Microbiology, *80 E. Concord St., Boston, Massachusetts*, 02215, United States of America

The long-term objective of this laboratory work is to identify nutritional factors which contribute to the high incidence of colon cancer in Western culture. In this proposal, studies are focused on the role of dietary and endogenous cholesterol and its metabolites in the gut lumen in large bowel tumorigenesis in an experimental animal. Continuing studies are planned on the interrelationship between dietary polyunsaturated fat and saturated fat, particularly as it relates to the disbursement of cholesterol systemically and/or enterally. Various nutritional regimens containing 20% fat with and without cholesterol are fed to rats given an indirect acting carcinogen, 1,2 dimethylhydrazine (s.c. or orally), or a direct acting carcinogen, N-methyl-N'-nitro-N-nitroguanidine (intrarectally). These diets will allow an evaluation of the interrelationships between the quality of dietary fat, the enteral excretion of cholesterol and its metabolites and large bowel tumorigenesis. Effects of cholesterol and its metabolites will be evaluated in terms of tumor number, histological types and location, latency period and tumor incidence. An assessment of fecal excretion of neutral and acid sterols as well as fecal 7-alpha-dehydroxylase and cholesterol dehydrogenase activities occurring in rats fed the various dietary regimes will permit correlative studies with large bowel tumorigenesis. Using the Ames Salmonella/microsomal assay for mutagens/carcinogens, various fecal sterol metabolites will be evaluated to pinpoint those components which may be carcinogenic or cocarcinogenic for further testing. These studies should provide: 1) a better understanding of the relationships between nutrition, microflora and colonic cancer; 2) additional information concerning the nutritional linkage between coronary heart disease and colon cancer and 3) additional guidelines for studies in human nutrition aimed at decreasing the incidence of colon cancer.

147. ENVIRONMENTAL CARCINOGENS AND CANCER OF THE GASTROINTESTINAL TRACT

Renwick, A. G., University of Southampton, Pharmacology Group, *Southampton SO9 5NH, England, United Kingdom*

OBJECTIVE: To determine the extent to which polycyclic aromatic hydrocarbons interact with the gut wall and how this interaction, i.e., metabolic activation and DNA binding, may be affected by the diet.

APPROACH: The concentrations of (3H)benzo(a)pyrene in the wall of the stomach, intestine and colon and its binding to DNA at such sites are measured following oral administration to normal rabbits and to animals fed high-fat diets. The effect of bile salts on the extent of binding is studied by measuring the binding to the stomach after addition of the labelled substrate to dietary lipid containing varying proportions of bile salt. The effect of high-lipid diets on the biliary excretion of the labelled polycyclic is also investigated.

The metabolism of benzo(a)pyrene and its liver metabolites by fecal microorganisms is studied by HPLC to determine the extent to which inactive conjugates may be reactivated in the colon. The metabolic activation of benzo(a)pyrene by the intestinal and colon wall is studied using microsomal fractions collected from normal rabbits, rabbits fed high-fat diets and rabbits fed benzo(a)pyrene with normal and high-fat diets.

148. MAMMARY CARCINOGENESIS - DIETARY FATS AND ANTIOXIDANTS

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The summary for this project is under entry no. 68.

149. DIETARY FAT, PROLACTIN AND MAMMARY CANCER

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This study will demonstrate the effects of long-term ingestion of high fat diets on serum prolactin and on induced and spontaneous mammary tumors in rats. The relationship between fatty acid composition and hormonal and tumorigenic effects will be defined. Evidence that ingestion of a high fat diet enhances rat mammary tumorigenesis will be assessed, employing DMBA-induced tumors incidence in rats fed one of several dietary fats. Natural dietary fats of widely varying fatty acid composition will be fed in a purified diet; serum prolactin will be monitored by RIA, and DMBA-induced and spontaneous mammary neoplasms will be identified. The possibility of predicting ultimate tumor response of animals from serum prolactin profiles will be determined by comparison of periodic serum prolactin values at estrus or diestrus with tumor development. The distribution of prolactin receptors in various mammary lesions will be determined by autoradiographic procedures. Dietary fat effects on the pathogenesis and incidence of spontaneous or induced benign or malignant mammary tumors will be evaluated separately rather than considering effects on all mammary tumors together.

This application requests supplementary funds to purchase a gamma counter for use in the prolactin assays.

150. NUTRITIONAL AND ENVIRONMENTAL VARIABLES IN THE ACTIVATION OF CARCINOGENS

McLean, A. E., University of London, University College Hospital Medical School, Dept. of Clinical Pharmacology, *University St., London WC1E 6JJ, England, United Kingdom*

OBJECTIVE: To measure dietary effects on enzymes concerned with activation and inactivation of carcinogens, to see whether the variation in national cancer patterns can be attributed to dietary effects on particular enzymes.

APPROACH: a) Rats are fed diets containing different kinds of fats. Benzo-a-pyrene hydroxylation, cytochrome P450, and glutathione transferase are measured, and the covalent binding of carcinogens is measured, using isolated liver cells. b) Plasma from rats fed different diets stimulates benzo-a-pyrene metabolism in isolated liver cells to a varying extent. This approach will be extended to human plasma samples.

151. DIETARY FAT IN COLON CARCINOGENESIS

Newberne, P. M., Nauss, K., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

This project is examining the effects of quality and quantity of fats on the induction of colon cancer in rat by the direct acting carcinogen NMU and the indirect carcinogen DMH. Ancillary studies include immunocompetence and metabolism in animals developing colon tumors under these conditions.

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152. THE ROLE OF LIPOTROPES IN CARCINOGENESIS

Poirier, L. A., Hyde, C., Mikol, Y., Wilson, M. J., Myasishcheva, N. V., Wenk, M., Ward, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Nutrition & Metabolism Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The mechanisms behind the alteration of chemical carcinogenesis by the dietary lipotropes, choline, methionine, folic acid and vitamin B12 have been studied. The metabolism and carcinogenic activity of ethionine in different species is being compared. Ornithine decarboxylase, a marker of tumor promotion and a precursor enzyme for the polyamines, is used to study the possible role of methyl insufficiency in tumor promotion. The great drop in growth rates of transformed liver epithelial cells placed in methionine-deficient, homocysteine-supplemented medium is due to their elevated growth rates in complete medium, rather than to an inability to synthesize methionine.

BIBLIOGRAPHIC REFERENCES: Wilson, M. J. and Poirier, L. A.: Increased methionine requirements of transformed liver cells in vitro. Exp. Cell Res. 111: 397-400, 1978.

153. CHARACTERIZATION OF BIOLOGIC ACTIONS OF ACRYLONITRILE

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This research proposal is a multidisciplinary approach aiming to characterize the biologic actions of acrylonitrile and to gain insight into its mechanism of action. The acute and chronic toxicity of acrylonitrile, and its saturated analog, propionitrile, will be studied in rats, mice, guinea pigs, hamsters and rabbits by using LD50 determinations, gross, light and electron microscopic observations, as well as biochemical and physiological measurements. The chemicals will be administered p.o. (gavage), i.v. or by inhalation in acute experiments; and put in drinking water or administered by inhalation in chronic assays. Specific attention will be paid to the neuroendocrine, gastrointestinal and pulmonary systems. The carcinogenic potential will be sought in male rats kept for 1, 2 or 3 years on drinking water containing acrylonitrile and Purina Lab Chow or lipotrope-deficient diets which were shown to enhance the tumorigenic action of several chemical carcinogens. The site of injury and severity of lesions will also be investigated after induction of mixed function oxidase, by diets, and by modification of hormonal states of animals. An important part of the project will be the identification of metabolites and tissue and subcellular distribution of radiolabelled acrylonitrile. Nucleic acid, protein and lipid fractions will be isolated in binding site studies. Alterations induced by acrylonitrile in tissue glutathione concentrations would probably yield data on the importance of GSH as a binding site of acrylonitrile or its metabolites and as a protective nucleophile. The multidisciplinary approach, concentrating on general toxicity experiments, neuropathologic, endocrine and gastrointestinal lesions, carcinogenic action, and distribution of acrylonitrile will characterize the biologic action of the compound and further our understanding of the mechanism of action and public health implications of this important chemical.

154. EFFECTS OF DIETARY BRAN ON COLON CANCER IN MICE

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Epidemiological studies have consistently reported the correlation of colon cancer incidence with diet. In particular, the positive correlation of high dietary fat with colorectal cancer is well documented and the mode of action is, at least, partly understood. However, any consistent negative correlation of dietary fiber with colon cancer incidences remains to be identified. Increases in dietary fiber are known to alter several physiological parameters by binding water, decreasing transit time, increasing fecal volume, binding bile acids and altering the intestinal microflora. Whether these changes exert a truly protective effect against colon cancer or the correlation simply reflects changes in fecal volume has yet to be determined.

Therefore, this study was designed to investigate the effect of specific well-characterized brans (soybean, corn, hard spring wheat, and soft winter wheat) on the tumor incidence in mice treated with 1,2-dimethylhydrazine (DMH). Beginning at 11 weeks of age, we inject barrier-derived male BALB/c mice subcutaneously weekly for 10 weeks with DMH (20 mg/kg body weight); this model was chosen because of the specificity for the production of distal colon and rectal tumors without tumors arising in other tissues. The low fat diets containing 20% bran are highly controlled being commercially synthesized to our specifications. Diets are given to mice beginning at 8 weeks of age and continuing throughout the experimental period until sacrifice.

At time of sacrifice (10 months after first DMH injection), mice will be killed; colons will be inflated with Telly's fixative, cleared and stained. Tumors will be identified through a dissecting scope, sectioned and stained and examined histologically. Groups will be compared and diets will be evaluated regarding their effects on tumor incidences, malignancy, and tumors per tumor-bearing mouse.

155. INFLUENCE OF DIETARY SELENIUM ON PANCREATIC CANCER

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The effects of selenium as sodium selenite fed at levels below the dietary requirement, near the requirement, and considerably above the requirement are being evaluated on the pancreatic cancer model induced by N-nitroso bis (2-oxypropyl) amine (BOP) in the Syrian hamster. Initially the three levels were selected by feeding hamsters excessively high levels of sodium selenite (0.25 up to 80.0 ppm Se) in a three week study and excessively low levels (0.0 up to 5.0 ppm Se) in a 25 week study. The selenium status of all animals was evaluated by growth; food consumption; blood and tissue selenium content; blood glutathione and glutathione peroxidase activity; and histopathology. In chronic studies with BOP the high (5.0 ppm) and low (0.0 ppm) levels of selenium are being fed to groups of hamsters either only before, only after or both before and after a single injection of BOP with the control level (0.10 ppm) fed during the remaining period. In addition, rats which develop colorectal tumors when treated weekly with BOP, are being fed control (0.10 ppm) or high (2.0 ppm) levels of selenium to observe the influence of selenium on this tumor model with BOP. In both chronic studies blood will be collected at several intervals for the determination of blood selenium, glutathione peroxidase and glutathione concentrations. The effects of dietary selenium on the BOP-induced alkylation of pancreas DNA, and the ability of DNA to repair are being used to further assess the effects of selenium on BOP-induced carcinogenesis. In addition to evaluating pancreatic histology, numerous other tissues will be evaluated in both BOP treated and their respective saline treated controls to evaluate the long term effect of feeding various levels of selenium on the longevity and general pathology of the Syrian golden hamster.

156. ALTERATIONS IN SELENIUM REQUIREMENTS BY DIETARY ANTAGONISTS

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OBJECTIVE: Diet is an integral factor in the overall occurrence of cancer in man. The possibility that reduced availability of selenium may increase the susceptibility of humans to diseased states remains to be determined. Epidemiologically, selenium consumption has been shown to be negatively associated with tumor incidence at all sites. Recent epidemiological evidence also suggests that other trace elements may likewise influence the susceptibility of individuals to the cancer state by possibly altering selenium availability.

APPROACH: These studies wil focus on the use of two model systems for determining the effect of selenium and potential selenium antagonists upon the enhanced development of tumorous tissue. The two model systems are the transplantable Ehrlich ascites tumor cell and colon cancer chemically induced by 1,2-dimethylhydrazine. Experiments will be conducted to determine if the form of selenium administered alters the susceptibility to various seleno-antagonists as measured by neoplastic tissue growth. These studies propose to use tumor cell growth as an indices of mineral interaction. These data would be of considerable value in the overall evaluation of diets in the etiology of cancer and the potential benefits of selenium on the growth of normal and neoplastic tissue. Experiments are planned to examine the possible mechanism by which selenium retards tumor growth.

157. EXPERIMENTAL PANCREATIC CARCINOMA - MODULATING EFFECTS OF GASTROINTESTINAL POLYPEPTIDE HORMONES AND ZINC

Barrowman, J. A., Pfeiffer, C. J., Memorial University of Newfoundland, School of Medicine, *Health Science Complex, St. Johns, Newfoundland,* A1C 5S7, Canada

The summary for this project is under entry no. 119.

158. DIETARY MODULATION OF EXPERIMENTAL PROSTATIC CANCER IN RATS AND OF RELATED HORMONAL STATUS IN RATS AND HUMAN SUBJECTS

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OBJECTIVE: The aim of this project is to study the modulating effects of selected nutritional factors (fat, protein, carbohydrate, caloric intake, vitamins A, E, selenium and zinc) on the development of chemically induced prostatic adenocarcinoma in the rat, and to study the possible value of these factors as a direct form of (prostatic) cancer therapy. The involvement of the endocrine system as mediator of possible dietary effects will be studied as well. To estimate the relevance of results of animal experiments for the human situation, the nutrition-hormonal status studies will be extended to human subjects.

APPROACH: Prostatic cancer in rats is induced by i.v. administration of 7,12-dimethylbenz(a)anthracene. Different diets are fed to groups of rats comparing low and high fat, protein, caloric intake and comparing degree of saturation of fats, origin of protein and oligo- and polysaccharides. These diets are fed to rats in which tumors will be induced or are induced and to intact rats as well as to human subjects. Hormonal status (androgens, estrogens, prolactin) will be determined by radioimmunoassay methods.

159. MODIFICATION OF THE DMH INDUCED ACUTE RESPONSE OF COLONIC EPITHELIAL CELLS

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The acute response of colonic epithelial cells to 1,2-dimethylhydrazine delivered subcutaneously at a weekly dose level of 20 mg/kg body weight to CF1 mice has been shown to induce a permanent alteration in the distribution of the major zone of DNA synthesis within some crypts one week following the 5th treatment. This coincides with the time when areas of focal atypism or microscopic adenomas are found. This proposal seeks to determine firstly if this response by colonic epithelial cells is elicited only by an organotropic carcinogen or if weekly injections of alkylating agents and antimetabolites such as cytoxan, bleomycin, 5-fluorouracil, hydroxyurea, and cytosine arabinoside induce similar alterations in cell proliferation. Secondly, mouse strains resistant to DMH (AKR/J, GR and DMA/2) will be examined and the acute effects of DMH on their colonic epithelial cells compared with that found in the sensitive CF1 strain. The effect of the ultimate carcinogen of DMH activation

of MAM on both DMH sensitive (SWR/J and P/J) and resistant strains will also be studied, as well as the effect of pyrazole administered prior to MAM. Lastly, substances such as selenium, zinc, B sitosterol, lithocholic acid, deoxycholic acid, and Vitamin C will be delivered prior to and during DMH and MAM treatment to determine if any modification of the acute reaction takes place and at which stage of the damage and repair cycle these dietary additives may operate.

160. INFLUENCE OF ETHANOL ON CARCINOGEN ACTIVATION

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OBJECTIVE: Epidemiological studies have demonstrated an increased risk of cancer in heavy consumers of ethyl alcohol. The research of this proposal will examine interactions occurring between ethanol and the metabolic activation of chemical carcinogens.

APPROACH: Isolated perfused rat liver will be the primary model for these experiments, because the activation, conjugation and binding of carcinogens to DNA may be studied simultaneously under physiological conditions. The influence of chronic ethanol consumption and acute ethanol administration on carcinogen activation will be studied. Direct effects of ethanol on enzymes of mixed-function oxidation and conjugation will be differentiated from indirect effects caused by the metabolism of ethanol and subsequent interactions with other pathways involved in carcinogen metabolism. The effects of acute and chronic administration of carcinogens on ethanol metabolism will also be determined in order to define points of interaction and the mechanisms involved.

161. ANTIMUTAGENIC AND PROMOTING ACTIVITIES OF FOOD SYSTEMS AND THEIR METABOLITES ON ENVIRONMENTAL MUTAGENS AND CARCINOGENS (ABBREV)

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Sublethal mutagenic and carcinogenic capacities of a compound are recognized as a health hazard. They are the basis of regulatory action. Less attention has been given so far to the health hazard of agents which enhance or promote the action of 'proper' mutagens and carcinogens. This group of compounds, including comutagens, promoters and DNA repair inhibitors, can enhance by several hundred fold mutagenesis and carcinogenesis.

The main research objectives are: (1) to examine and quantitate the comutagenic (cocarcinogenic), antimutagenic (anticarcinogenic), and promoting activity of food systems, food components, and reaction products on the mutagenic/carcinogenic effect of environmental mutagens/carcinogens which represent four different molecular groups; (2) to examine possible enhancing or inhibiting interactions between mutagens and their metabolic derivatives and comutagens (antimutagens) and their metabolic derivatives; (3) to design and validate simple, economic tests for the detection of comutagens, antimutagens and promoters in food products; (4) to simulate in vitro those conditions which prevail in mammals including man, and (5) to initiate experiments to uncover the role of antioxidants on the level of mutagens in the urine and feces of human population groups at high cancer risk (smokers).

162. DIET AND ITS EFFECT ON ENZYMES LINKED TO COLON CANCER

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Previous studies in our laboratory have implicated fecal bacterial enzymes in the activation of procarcinogens in the intestine. By suppressing the metabolic activity of the microflora with diet (low fat), Lactobacillus feeding, or antibiotics, a reduction in the incidence of dimethylhydrazine-induced colon tumors in Fischer rats was observed. Our current plan is to administer procarcinogens and marker compounds by the oral and intracecal routes. The formation of metabolic products and mutagens will be assayed in urine and feces, and will be compared to the fecal bacterial enzymes, beta-glucuronidase, nitroreductase and azoreductase. The procarcinogens are 4-dimethylaminoazobenzene - a substrate for azoreductase. 4-amino-2', 3-dimethylbiphenyl-glucuronide - the glucuronide of an intestinal carcinogen; hydrolysis is mediated by beta-glucoronidase, beta-naphthol-glucuronide - a substrate for beta-glucuronidase; closely related to the carcinogen naphthylamine; a marker compound, naphthol-glucuronide, will test fecal beta-glucuronidase. We will alter the metabolic activity of the microflora by the following maneuvers: diet (low vs. high fat); administering antimicrobial agents (tetracycline and metronidazole); feeding Lactobacillus supplements; and giving sacchrolactone, a specific inhibitor of beta-glucuronidase. The effect of these procedures can be assessed rapidly by measuring metabolic products of the procarcinogens, mutagens and bacterial enzymes in the feces. Studies in humans will attempt to suppress fecal bacterial enzymes by feeding Lactobacilli and by giving a low dose of tetracycline. Two marker compounds will be used to determine the activity of fecal enzymes: salicylazosulfapyridine, a substrate for azoreductase, and phenolphthalein-glucuronide, a substrate for beta-glucuronidase. It is hoped that these studies will provide a useful method to assess the role of the intestinal microflora in activating several classes of procarcinogens, as well as investigating strategems to suppress bacterial metabolism in animals and humans.

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163. CELLULAR ASPECTS OF MUTAGENESIS AND CARCINOGENESIS

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This project seeks to understand human susceptibility to mutagenesis and carcinogenesis by chemicals from the utilization of coal for energy production. It focuses on the damage to DNA and is based on the hypothesis that this damage gives rise to the initiation or mutagenesis and carcinogenesis. We are obtaining evidence that cellular susceptibility to chemical carcinogenesis is modulated in three ways: (1) the cell is often involved with metabolism of a carcinogen to either detoxification products or to compounds more active in carcinogenesis, and this metabolic potential varies among cell types, and among similar cells exposed to different dietary regimes; (2) alterations in chromatin structure can regulate the availability or critical site for binding a carcinogen as well as the repair efficiency along the DNA; and (3) damage remaining in the DNA at the time of replication is most detrimental to the cell and different cell types have different capacities to replicate DNA in the presence of this damage.

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164. EFFECT OF NUTRITION ON CARCINOGEN AND TOXIN METABOLISM (RAT)

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This is part of a broader project. A summary of this subproject is not available.

V. OTHER STUDIES OF MODIFICATION OF CHEMICAL CARCINOGENESIS IN MODEL SYSTEMS

165. MODIFIERS OF CHEMICAL CARCINOGENESIS IN CELL CULTURE

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Humans are constantly exposed to the vast mixture of chemicals that are present in our environment. Often this mixture (e.g urban air) contains both carcinogenic polycyclic aromatic hydrocarbons and other types of polycyclic hydrocarbons that can act both as cocarcinogens with some carcinogenic hydrocarbons and as inhibitors of carcinogenesis with others. In addition, mixtures such as cigarette smoke contain other classes of chemicals which can alter the effects of exposure to carcinogenic polycyclic hydrocarbons by acting as cocarcinogens or as tumor promoters. To determine the mechanism by which certain hydrocarbons can potentiate the carcinogenicity of some carcinogenic hydrocarbons and inhibit cancer induction by others, their effects on the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons and the induction of biological effects will be measured in hamster embryo cell cultures. These studies will include quantitative measurements of hydrocarbon oxidation, conjugation, DNA-interaction products and the induction of cytotoxicity, cell-mediated mutation and transformation. The role of conjugation of hydrocarbon metabolites to glucuronic acid in the induction of biological effects will be examined. The effects of other types of modifiers such as cocarcinogens of other classes and tumor promoters on the above parameters will also be measured. The results obtained will be of use in predicting how the effects of exposure to carcinogens are altered in the presence of hydrocarbons and other types of cocarcinogens and tumor promoters. This will permit modification of these effects and aid in the development of short term assays with enhanced sensitivity for the detection of carcinogens in the environment.

166. TEMPORAL ASPECTS OF TUMORIGENIC RESPONSE TO INDIVIDUAL AND MIXED CARCINOGEN EXPOSURES IN RELATION TO RISK ASSESSMENT

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The research is designed to obtain a better understanding of the temporal kinetics of tumor induction when one or more carcinogens are present simultaneously or sequentially for prolonged periods of time. Studies done to date under this contract have shown that carcinogens in mouse skin by polycyclic aromatic hydrocarbon carcinogens is consistent with the induction of dependent and autonomous cell transformation by the carcinogen followed by the conversion of autonomous tumor cells into malignancies at a rate which is determined by the level of carcinogen exposure. Dependent cell transformations remain latent in the skin unless expressed by a promoting agent. Dependent neoplasia appear to follow one-hit kinetics while malignancy is a multihit endpoint. Dose-related and time-related aspects of tumor induction are separable in the initiation-promotion system of mouse skin used as a model. Tests of the initiating activity of betapropriolactone, 4-nitroquinoline-oxide, and ultraviolet light have been made. The dose response for promoter activity has been completed and indicates a nonlinear relationship. Prenatal initiation indicates that material exposure can produce the same type of promotable tumors as occur in postnatally initiated mice. Stop experiments have indicated that mice exposed to a combination of an initiator and a promoter develop autonomous benign tumors that readily progress to carcinoma even though the carcinogen is no longer being applied. Antipromoting retinoids were tested against polycyclic hydrocarbon-induced mammary tumors in rats and it was found that their activity was effective even in the earliest states of carcinogenesis when the carcinogen or its metabolites were still available to the target cells. In hamster lung no cocarcinogenic effct of ferric oxide was found when applied with benzo(a)pyrene.

BIBLIOGRAPHIC REFERENCES: Pereira, M.A., F.J. Burns and R.E. Albert, Dose-Response for Benzo(a)pyrene Adducts in Mouse Epidermal DNA, Cancer Res. 39: 2556-2559 (1979).

167. CARCINOGENESIS STUDIES IN CULTURED COLON FROM EXPERIMENTAL ANIMALS AND HUMANS

Autrup, H., Harris, C., Haugen, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Human Tissue Studies Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A model system for studying chemical carcinogenesis in colon has been developed. Human colon can generally be maintained 14 days and rat colon at least 63 days as explant culture. Cultured human and rat colon have the ability to enzymatically convert various classes of chemical carcinogens, such as polycyclic aromatic hydrocarbons, N-nitrosamines, mycotoxins and hydrazines, into metabolites which react with cellular macromolecules, such as DNA and protein. A wide variation among people in the binding of both benzo(a)pyrene (BP) and 1,2-dimethylhydrazine (DMH) was seen. The data showed a skewed distribution which strongly suggested a distribution more complex than uni-modality. The carcinogen-DNA adducts for aflatoxin B1 (AFB), BP, N-nitrosodimethylamine and DMH have been identified and the persistence of the AFB-DNA modification has been studied in human colon. The effects of various suspected inhibitors and enhancers of colon carcinogenesis on the metabolism of BP and DMH have been investigated.

BIBLIOGRAPHIC REFERENCES: Autrup, H., Schwartz, R.D., Essigmann, J.M., Smith, L., Trump, B.F., Harris, C.C.: Metabolism of aflatoxin B1, benzo(a)pyrene and 1,2-dimethylhydrazine by cultured rat and human colon. Teratogenesis, Mutagenesis and Carcinogenesis, in press, 1980. Autrup, H.: Explant Culture of Human Colon. In Harris, C.C., Trump, B.F., and Stoner, E.D. (Eds.): Cultured Human Tissues and Cells in the series Methods in Cell Biology. New York, Academic Press, 1980, Vol. 21B, pp. 385-401.

168. PANCREATIC CARCINOGENESIS - ROLE OF DUODENAL REFLUX

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To determine: 1) What physiologic or pharmacologic events or chemicals influence pressure relationships between the duodenum and pancreatic duct which might favor reflux of duodenal contents into the pancreatic duct; and 2) if carcinogens can reflux from the duodenum into the pancreatic duct and induce pancreatic carcinogenesis, we have established a canine model. In the past year we have: 1) Demonstrated that during fasting pancreatic and biliary secretion into the duodenum are cyclic and associated with interdigestive motor activity; 2) detected small amounts of duodenally perfused marker within the pancreatic duct in a minority of dogs during fasting; 3) determined that hormones (secretin and cholecystokinin/octapeptide) maintain or cause a fall in the pancreatic duct pressure to duodenal pressure gradient; 4) demonstrated that nicotine diminishes the cycling time of both the secretory and motor component of the interdigestive motor complex; and 5) in preliminary studies found that 14C-benzanthracene is secreted into the gastrointestinal tract cyclically in concert with the secretory and motor cycles of the interdigestive motor complex. These preliminary results suggest a link between cigarette smoking and pancreatic carcinogenesis. If carcinogens are cyclically secreted into the gastrointestinal tract in concert with gastrointestinal motor and secretory activity and nicotine diminishes cycling time, nicotine may increase exposure of the gastrointestinal tract to carcinogens.

BIBLIOGRAPHIC REFERENCES: DiMagno, E.P., Hendricks, J.C., Go, V.L.W. and Dozois, R.R.: Relationships among canine fasting pancreatic and biliary secretions, pancreatic duct pressure and duodenal phase III motor activity -Boldyreff revisited. Dig. Dis. Sci. 24:689-693, 1979. Keane, F.B., DiMagno, E.P., Dozois, R.R., and Go, V.L.W.: Relationships

among canine interdigestive exocrine pancreatic and biliary flow, duodenal motor activity, plasma pancreatic polypeptide, and motilin. Gastroenterology 78:310-316, 1980.

169. MODIFICATION OF CHEMICALLY INDUCED AND SPONTANEOUS CANCERS BY ENVIRONMENTAL AND OCCUPATIONAL STRESSORS (ABBREV)

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The aim of this project is to investigate the influence of environmental and occupational stressors (crowding, isolation, light and noise, moderate hypo- and hyperthermia) combined with ionizing and non-ionizing (microwave) radiation on development and growth of experimental cancers. Painting of skin with 3,4-benzopyrene resulting in development of skin cancer will be employed, and strains of mice with high incidence of spontaneous breast cancer (C3H) will be used as experimental models.

Treatment with 3,4-benzopyrene will be applied at different periods prior to or simultaneously with exposition to the above stressors or in combination with radiation. Time of tumor development (7-range macroscopic scale) and survival of animals will be evaluated.

Natural antineoplastic resistance (lung cancer colony test) and natural killer activity in animals exposed for different periods to the above stressors will be also evaluated.

170. THE EFFECT OF STRESS ON CARCINOGENESIS OF SALIVARY GLANDS

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The influence of hormones and pharmacons on salivary glands was studied by different authors.

This work was carried out to observe if stress induced by cold, 15 degrees Celsius, 10 min per day, was able to modify time or characteristics of carcinogenesis of salivary glands induced by DMBA. The action of a stress stimulus does not modify any histological aspect of normal rats in spite of the fact that the levels of plasmatic corticosterone in these experimental animals were higher than the normal levels in these animals.

The data pertaining to experimental carcinogenesis and stress are being computed at present.

171. PATHOMECHANISM OF TEMPERATURE CHEMICAL-CARCINOGENESIS

Weisbrode, S. E., Weiss, H. S., Ohio Agricultural Research & Development Center, *Columbus, Ohio,* 43216, United States of America

OBJECTIVE: Morphologically evaluate the effect of temperature on the development of chemically-induced squamous cell carcinoma.

APPROACH: Morbid anatomy, light microscopy and electron microscopy will be used to evaluate the pathoanatomic mechanisms by which normal, elevated or reduced ambient temperature effect benzopyrene induced squamous skin cell carcinomas.

172. COCARCINOGENESIS

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The goals of this project are designed to provide information with which to estimate the contribution of physiological stress to the process of transformation and malignancy. Model systems consisting of rodents as well as cell cultures are being used to evaluate the modification of normal host physiology on subsequent cancer occurrence. In particular, tumor promoters, exposure to x-ray and ultraviolet light are being investigated.

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173. RESEARCH PROGRAM ON THE REGULATION OF CELL CYCLES OF NORMAL AND CANCEROUS MAMMARY CELLS

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The research programs to be pursued during the tenure of this award will involve primarily the study of agents which may regulate the cell cycles of normal and cancerous mammary cells. Studies are planned in which the contributions of the polyamines, prostaglandins and cyclic nucleotides to the cell cycles of synchronized mammary cells will be determined. Cells to be studied include the human MCF-7 mammary cancer cells and cells from DMBA-induced tumors of rats.

174. GENETICS OF AFLATOXIN METABOLISM AND ITS ROLE IN CARCINOGENESIS

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The proposed research involves investigations of the genetic regulation of aflatoxin B1 (AFB1) metabolism via activation and deactivation pathways; the effects of environmental factors, i.e., polycyclic aromatic hydrocarbons (PAHs), that determine the genetic regulation of AFB1 metabolism in mice on the metabolism of AFB1 by humans; and the role of the genetics of AFB1 and inducers that cause induction or the repression of the specific pathways of AFB1 metabolism. The objectives of these studies are: (a) to understand the genetic regulation of AFB1 metabolism via various pathways, including activation and detoxification; (b) to develop genetic models for studies on aflatoxin carcinogenesis and toxicity; (c) to define the role of the genetic regulation of AFB1 metabolism via various pathways in the determination of susceptibility to aflatoxin B1; and (d) to study the effect of smoking on the metabolism of AFB1 by placental tissues obtained after delivery.

Much of the proposed work will be performed (a) in vitro with labeled AFB1, DNA, hepatic microsomes and other subcellular fractions isolated from rodents, and human placental tissue, and (b) in vivo with different strains of rats and mice for the purpose of investigating the correlation between in vitro metabolism and in vivo metabolic activation in an effort to assess the role of the genetics of AFB1 metabolism in toxicity and carcinogenesis.

175. AGE-RELATED CHANGES IN CHEMICAL CARCINOGENESIS

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The ability of the liver of 5- to 27-month-old male Fischer F344 rats to activate and metabolize aflatoxin B1 (AFB1) was studied. The conversion of AFB1 to mutagenic compounds by liver homogenates was determined using the Salmonella/microsome test. The liver homogenates from 12-month-old rats produced twice as many revertants as liver homogenates from either 5-, 18-, or 27-month-old rats. There was no significant difference in the ability of liver homogenates from 5-, 18-, or 27-month-old rats to activate AFB1. A comparison of the ability of liver homogenates from 5-, 12-, and 20-month-old rats to form AFB1-DNA adducts was made also. Liver homogenates from 12-month-old rats produced twice as many adducts as liver homogenates from 5- or 20-month-old rats. The conversion of AFB1 to water-soluble and chloroform-soluble metabolites by liver homogenates was studied also. An age-related change in the conversion of AFB1 to AFQ1 was noted.

The ability of liver homogenates from 5- to 30-month-old female Fischer F344 rats to activate AFB1 was also studied. In contrast to male rats, the activation of AFB1 was lowest by liver homogenates from 12-month-old female rats. Therefore, the ability of the liver to activate AFB1 appears to change with age in Fischer F344 rats, and the age-related change appears to be different for male and female rats.

176. BLADDER CANCER - METABOLISM OF CARCINOGENS AND PREVENTION

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The objectives of this research proposal will be: a) identification of products of N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide (FANFT) and 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT) cooxidative metabolism; b) investigation of the mechanism of FANFT and ANFT cooxidative metabolism by prostaglandin endoperoxide synthetase (PES); c) determination of reactive intermediates formed during FANFT and ANFT cooxidative metabolism; d) determination of the distribution of renal FANFT deformylase activity; and e) determination of the in vivo effects of PES-mediated cooxidation on the incidence of chemically-induced urinary bladder cancer using feeding studies.

Cooxidative metabolism will be defined as metabolism which is initiated by specific substrates and blocked by specific inhibitors of PES. Renal inner medullary microsomes and slices will be used to characterize cooxidative metabolism. Metabolites will be purified by gas liquid and high pressure liquid chromatographic techniques and identified by gas chromatograph mass spectral analysis. Metabolic requirements will be used to determine whether the fatty cyclooxygenase or prostaglandin hydroperoxidase activities of PES are involved in cooxidation. Radiolabeled FANFT and ANFT will be synthesized.

The effect of aspirin or indomethacin, specific inhibitors of cooxidative metabolism, on the incidence of FANFT-induced bladder cancer will be examined. Urinary cooxidative metabolites of FANFT and ANFT will be identified. Urinary prostaglandin E2 will be analyzed by a specific radioimmunoassay technique. This proposal will provide further insight into the genesis of chemically-induced urinary bladder cancer and a more rational approach to its prevention.

177. PREVENTION OF FANFT URINARY BLADDER TUMORIGENESIS

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The overall objectives of this project are to study the mechanisms by which carcinogens induce bladder cancer and to study factors which may intervene in these mechanisms to prevent such cancers. The interaction of N-(4-(5-nitro-2-furyl)-2-thiazolyl) formamide (FANFT), labeled by conjugation with an azo dye, with DNA and polynucleotides will be studied in vitro. The effects of allopurinol and 2,5-di-O-acetylglucosaccharo-(1, 4) (6, 3)-dilactone on the carcinogenicity of N-butyl-N-(4-hydroxybutyl) nitrosamine will be evaluated. The influence of these two compounds on tissue distribution and macromolecular binding of (14C) FANFT in rats will be determined. Rats will be fed uracil to produce urinary stones, and the effect of these stones on the induction of bladder cancer by FANFT will be ascertained. Harmane, an element of charred meat and a known comutagen, is thought to be a metabolite of tryptophan. Harmane's ability to promote bladder carcinogenesis will be studied. Two mutagenic hydroxamic acids, 2-naphthohydroxamic acids and benzoylaminoacetohydroxamic acid, will be fed to rats to assess their carcinogenicity. Finally, a series of polyphenolic compounds will be tested for mutagenicity. These studies may have relevance to the prevention of human bladder cancers in that they will contribute to the understanding of bladder tumorigenesis in experimental animals and in the identification of environmental carcinogens.

178. CHEMICAL FACTORS IN FETAL ONCOGENESIS

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In a recent study we found that 10 ppb dimethylnitrosamine given in the drinking water of strain A mice from preconception until 22 weeks of age caused a significant increase in lung tumors. We are repeating and extending this experiment to include extended periods of time, endpoints up to 28 months, so that effects of longer term exposure to this amount of DMN can be determined.

In a parallel investigation, we observed that treatment of pregnant mice with poly-chlorinated biphenyls resulted in a large increase in dimethylnitrosamine demethylase activity in the livers of their progeny before weaning. The effect was mediated largely by lactational delivery of the PCBs. In a tumorigenicity assay in progress, we are testing the effect of maternal treatment with PCBs on the carcinogenicity of DMN given to the offspring between birth and weaning. We also intend to measure DMN metabolism in other organs of the young mice, especially lung, pancreas, and kidney.

Another possible environmental agent, N6(methylnitroso)adenosine, has been found to be a multipotential carcinogen in the mouse. We are studying the mechanism of its metabolic activation by high pressure liquid chromatography by determining and quantifying the types of products formed by different organ and subcellular preparations, the effect of inhibitors, etc. DNA modification experiments will also be attempted.

179. ENVIRONMENTAL NITROSAMINES - METABOLISM & CARCINOGENESIS

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The metabolism and mechanism of action of the environmental carcinogens nitrosodiethanolamine, nitrosopyrrolidine, and nitrosomorpholine will be studied. Nitrosodiethanolamine occurs in the environment at higher levels than any other nitrosamine and nitrosopyrrolidine is also widely distributed; nitrosomorpholine can be formed readily by nitrosation in vivo.

Metabolic profiles for these nitrosamines will be developed with special emphasis on the pathways of metabolic activation. The role of alpha-hydroxylation in activation of these compounds will be studied, using newly developed assays for alpha-hydroxylase activity. The relationship of alpha-hydoxylation to organ specificity and the inducibility of alpha-hydroxylase activity by environmental modifiers will be determined. The effects of environmental modifiers on carcinogenesis by these nitrosamines will be studied by bioassays in experimental animals. Other possible activation pathways such as beta-hydroxylation and oxidation will be investigated with the aid of the nitroasamine specific Thermal Energy Analyzer; bioassays of nitrosamine containing metabolites will be conducted. These experiments are expected to provide a clearer understanding of nitrosamine metabolism and mechanism of action, an important goal in view of the widespread occurrence and carcinogenicity of these compounds.

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180. NEURO-ONCOGENESIS BY RESORPTIVE CARCINOGENS

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Alkylnitrosoureas, because of their chemical simplicity and known catabolism as well as their ability to induce a high incidence of neurogenic tumor with precision, provide an excellent opportunity to study the basic problem of neoplastic transformation. It is proposed to examine the formation and removal of O6-alkylguanine as a possible DNA lesion crucial to neoplastic transformation. Attempts will be made to correlate alterations in DNA and RNA synthesis and the metabolism of nonhistone chromosomal proteins induced by methylnitrosourea and ethylnitrosourea with the carcinogenic potential of these compounds. Gene activation associated with tumor promotion will also be studied in relation to proteolytic derepression by the chromosomally associated serine-protease. Induction of the synthesis of helix destabilizing protein and its control at the level of transcription or translation will be investigated as the process responsive to both tumor promotor and chemical carcinogen.

The precision of ethylnitrosourea in the neurogenic tumor induction will further be utilized in evaluating the role of nerve growth factor in suppression or regression of neoplastic transformation. The recently discovered thymic lymphoma production in rats exposed to methylnitrosourea will be studied in pursuit of establishing the tumor model for future biochemical, immunological and therapeutic investigations.

181. EXPERIMENTAL PATHOLOGY OF RENAL CARCINOGENESIS

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The objective of the proposed research is to identify the sequential stages of tissue and cellular events which occur during the induction of renal neoplasia by nitroso compounds and to develop further cell culture models which will provide information that will correlate directly with the in vivo process. Emphasis will be placed on the identification of target cells and their responses to carcinogens, and on the identification of precursor lesions and their progression, utilizing techniques of isotopic labeling and electron microscopy. Cell cultures derived from carcinogen-treated animals will provide simpler experimental systems facilitating the analysis of target cells in terms of their ultrastructural, proliferative, biochemical and functional characteristics and of the stages in the transformation process. Additionally, it is proposed to seek and test for modifying factors effective in the tumor systems and to investigate their mode of action in the pre-determined stages of renal tumor induction. The broad objectives of the project are to further the understanding of the basic biology of human renal neoplasia and of the process of chemical carcinogenesis in general, and to identify strategies which may reverse or inhibit the cancer process.

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182. CELLULAR AND BIOCHEMICAL EFFECTS OF TUMOR PROMOTERS

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The objective of this proposal is to elucidate cellular and biochemical effects of phorbol ester tumor promoters, and related compounds, and to determine the relevance of these effects to the mechanism of tumor promotion. We will test the hypothesis that tumor promoters act by usurping cellular receptors for endogenous growth controlling substances. We will also determine the structural and functional effects of the phorbol esters on membrane lipids and proteins. Effects of phorbol esters on differentiation and gene expression will be examined in cell culture systems and in developing organisms. The protein profile of cells exposed to phorbol esters will be studied to determine the possible roles of proteases, post-translational modifications or altered rates of de novo protein synthesis. The mechanism of interaction between initiating carcinogens, oncogenic viruses and tumor promoters in cell transformation will be studied in fibroblast and epithelial cell culture systems. The specificity of the effects observed in all of these studies, and their relevance to tumor promotion, will be tested through structure-activity correlations, the use of known inhibitors of tumor promotion, and comparative studies in cell culture and epidermal systems.

The results should enhance our understanding of the multistep nature of carcinogenesis and provide in vitro systems for detecting tumor promoters in the human environment.

183. TUMOR PROMOTERS AND CELL DIFFERENTIATION

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We have found that phorbol diesters with tumor-promoting activity in mouse skin reversibly inhibit terminal differentiation of erythroleukemia cells and preadipose cells in culture. We propose now to investigate the mechanism of the inhibition and the role that transient inhibition of differentiation by tumor promoters may play in carcinogenesis.

A series of differentiating cell lines will be examined to determine if tumor promoters inhibit differentiation only in cultures in which the differentiating cells have a limited life span and proliferative capacity (erythroleukemia, preadipocytes, myoblasts) or whether they also inhibit differentiation in cultures in which the differentiating cells retain the potential for continuous proliferation (myeloma, melanoma, colon adenocarcinoma). If the primary action of the promoters is inhibition of expression of 'luxury' genes (e.g., hemoglobin), we will determine if the block is at the level of transcription or translation. If the primary effect is to block entrance of cells into G0 (extended G1), the role of polyamines in the block will be examined.

We will determine if the frequency of induced mutations in differentiating populations is increased when cells are treated first with a mutagen and then with a promoter. To determine if initiation by a carcinogen can itself inhibit terminal differentiation or can 'lock in' the transient inhibition of differentiation by tumor promoters, 'normal' differentiating cells (3T3 preadipocytes) will be transformed according to one-stage (carcinogen alone) and two-stage (carcinogen plus promoter) protocols, and cells isolated from transformed foci will be analyzed for the capacity to differentiate.

It is becoming increasingly evident that cancer in humans is often the result of the interaction of environmental carcinogens and cocarcinogens such as tumor promoters. These studies on the relationship between cell differentiation and carcinogenesis will help to elucidate the mechanisms by which these interactions lead to neoplasia.

184. NEW APPROACHES TO SYSTEMIC TWO-STAGE CARCINOGENESIS

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The immediate aim of the proposed research is to extend our knowledge and the scope of systemic two-stage carcinogenesis. The ultimate purpose of the research is to enable application of standard analytical procedures to the separate stages of initiation and promotion in systemic carcinogenesis which have been so successfully applied in the past to skin carcinogenesis.

The proposed experiments may be considered under 2 headings: (1) The search for new initiators (e.g., tritiated thymidine) and potential promoters (e.g., various hormones) applicable to two-stage systemic carcinogenesis; and (2) the testing of new methods for two-stage systemic carcinogenesis. The latter include (a) transplacental carcinogenesis, with the initiator administered to the pregnant mother, and the promoter to the offspring after birth, and (b) the adaptation of intrasplenic transplantation of endocrine organs, to be performed in relation to feedback mechanisms via the pituitary, and the combined action of DMBA and results of intrasplenic transplantation.

185. EARLY AND CRITICAL EVENTS IN CHEMICAL CARCINOGENESIS

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While it is desirable to minimize human exposure to carcinogenic agents, the methods used to identify such agents should depend on endpoints other than the induction of human disease. The use of such methods, the less costly and time consuming the better, necessarily involves assumptions as to their relevance to the human condition. Research into the various aspects of the mechanism of chemical carcinogenesis can thus be said to have as a practical goal the elimination or validation of assumptions made in extrapolating from simple test systems to man. In our program we are specifically investigating the following aspects: (1) identification of the adducts formed when aromatic amine carcinogens attack nucleic acids in target tissues, and determination of the susceptibility of such adducts on DNA to repair; (2) theoretical methods for predicting adduct formation (prediction of rate, solvent competition, and product distribution); (3) identification of the immediate mutagenic consequences of each type of lesion; (4) determination of reasons for lack of tumorigenicity in tissues which are chemically attacked by carcinogens; (5) dissection of the initiation and promotion processes in tumor induction.

186. STUDIES ON THE METABOLISM OF CHEMICAL CARCINOGENS

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The metabolic pathways of chemical carcinogens, generally various aromatic amines, are studied both in animals and in isolated tissues. Metabolites are separated by various techniques such as thin-layer, column, gas-liquid, or high pressure liquid chromatography. Identification is made through physicochemical means such as mass spectrometry, nuclear magnetic resonance and ultraviolet spectra. Interaction of metabolites with cellular constituents is determined. The effect that inhibitors or promoters of the carcinogens exhibit on the metabolic patterns is studied.

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187. HEALTH SIGNIFICANCE OF FILTH

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STATEMENT OF OBJECTIVE: To demonstrate the health significance of foodborne animal and mold contaminants to the consumer.

DESCRIPTION OF WORK: 1. Conduct research on the isolation and identification of toxic water and fat extractables from insect contaminants of food. 2. Continue literature search on toxic and allergenic aspects of mite contaminants in stored foods. 3. Continue studies to evaluate the co-carcinogenic relationship of Geotrichum candidum and methyl-benzylnitrosamine using Ames assay technique. 4. Conduct research on the relationship of insect infestation in stored grains and subsequent mycotoxin production in the product.

188. CHRONOBIOLOGY AND OCCUPATIONAL HEALTH HAZARDS

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A systematic investigation of the influence of circadian and circannual rhythms on the effects of several toxic or carcinogenic chemicals commonly found in the workplace will be made with mice. Based on previous studies it is expected that a fixed dose at one circadian time will be lethal to 10% of the animals (LD10) or less but at another time to 80% (LD80) or more. For the purpose of distinguishing optimal conditions for tests at single or multiple time points, one or more model synchronizers will be manipulated to determine their relative roles: 1. The role of rhythms in influencing the outcome of toxicity tests will be tested; this will be done with emphasis on both the circadian and circannual frequencies. 2. From the viewpoint of circadian-stage dependence, carcinogens will be examined under a variety of conditions compatible with the usual working day of the laboratory. Time will be varied along either the presence or absence of a hormonal millieu such as a pituitary implant or a murine virus, both of which favor the genesis of breast cancer. Rhythms in temperature and other selected variables will be explored to see if they can be used as sensitive gauges or serve as harbingers of early pathology. 3. The relative strengths of competing synchronizers that influence results or that may be used to manipulate rhythms will be considered. Animals maintained under standard light-dark schedules (LD 8:16) will be compared to those subjected to staggered schedules with or without the additional influence of restricted feeding periods. The results should determine wether the now common practice of ignoring circadian and circannual variation may be continued and when multiple tests are required or when a single time point will suffice. The best conditions for obtaining maximal information in the most convenient manner will be decided. Thus, reliable methods are to be developed for carrying out chronobiological analyses in the context of a regular laboratory routine and without the necessity of undesirable schedules, increased staff or other unrealistic expenses. 4. The protocols we develop will be tested in other laboratories in this country and abroad.

189. EXPERIMENTAL RADIATION-INDUCED AND CHEMICAL-INDUCED

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It is commonly stated that 50 to 90 percent of human cancer may be the result of environmental factors and that of this environmental cancer a large portion may be the result of pollutant chemicals from energy production and use. Breast cancer in the human female usually has been ingored as an environmental cancer. There are at least two lines of evidence that support the concept that human breast cancer may be an environmental cancer. First, the increased incidence of breast cancer in Japanese women may be correlated with increasing industrialization and westernization of Japanese life-style. Secondly, chemicals thought to be model compounds for pollutant chemicals and/or chemical pollutants themselves may induce mammary neoplasia (and cancer) in the rat, where the rat mammary tumor system is used as a model for the human breast cancer situation. It is the intent of the present research to study, in a quantitative fashion, the relationship of pollutant chemicals, or model compounds of pollutant chemicals, to mammary neoplasia induction in female rats. The synergistic, additive or inhibitory interaction of these compounds, the effect of route of entry into the body, the influence of hormonal manipulation, and diet will be studied in this model system in an effort to assess the potential impact of energy pollutants upon breast cancer in the human female.

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190. ANALYSIS OF CELLULAR EVENTS IN CHEMICAL CARCINOGENESIS

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The major aim of these studies is to analyze and modify currently available models of chemical hepatocarcinogenesis, as well as to extend new models which we have developed; and to delineate obligate components in the sequence of alterations which result in the appearance of carcinomas. To accomplish this purpose, we will examine the phenomena of synergism between carcinogens; the mechanism of sensitization of carcinogen-action which results from cell division; and the influence on carcinogenic progression of a number of non-carcinogenic chemical and nutritional factors. For these purposes, we will utilize carcinogens which differ as to chemical class and differ in their mode of interaction at the molecular level. To determine the sequence of alterations thus induced, we will use a group of 'markers' which include histochemistry (morphologic), alpha-fetoprotein (biologic) and alkaline elution gradients (biochemical). The histochemical markers will include the enzyme gamma-glutamyl transpeptidase, a membrane enzyme which demonstrates early alteration during carcinogen exposure; iron uptake under conditions of iron overload; and a 'new marker,' nile blue sulphate, which stains for accumulation of fatty acids. Circulating alpha-fetoprotein will be followed as a re-expression of fetal function which has been demonstrated to be associated with various cell populations during carcinogenesis. Alkaline elution gradients will be utilized to examine alterations in the DNA of target cells. The use of these measurements will also be aimed at determining if alterations of differing type are independent or coordinated.

191. THE MECHANISM OF CELL TRANSFORMATION

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The conditions which are responsible for modulation in vitro transformation will be investigated further, with specific emphasis on the use of UV, X-rays, combination of carcinogens of different classes, the combination of in vivo - in vitro sys-

tems, and the effect of repair mechanisms on toxicity and transformation. The relevance of chromosome damage as a result of initial carcinogen insult as well as to which cytogenetic alterations are responsible for or associated with malignancy is being determined using cells from a variety of species including human. Studies on the mechanisms of early events of carcinogen-cell interaction will be continued, as well as investigation of different proximate carcinogens and metabolic inhibitors. Special emphasis will be given to the requirements necessary to obtain neoplastic transformation of human fetal cells. The testing for the tumorigenicity of cells exposed to different agents for different time intervals will continue.

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192. BIOCHEMICAL FACTORS THAT CONTRIBUTE TO ORGAN-SPECIFIC CARCINOGENESIS AND SELECTIVELY MODIFY SUSCEPTIBILITY OF FETAL TISSUES TO CARCINOGENESIS

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Factors that modify tissue responses to chemical carcinogens in different organ systems are studied to evaluate their contribution to changing susceptibilities to carcinogenesis in certain tissues during prenatal and postnatal development. Tissues and organ systems currently under study include the nervous system, kidneys, liver, and intestinal mucosa. Modifying processes now being studied include excision repair of DNA, transport of carcinogen into and within cells, and the changing cellular susceptibility to carcinogens during different stages of the cell cycle. Factors investigated in the past, and anticipated for future study, include immune responses to tumors, the induction and genetics of enzymes metabolizing carcinogens, and tumor promotion.

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193. STUDY OF CARCINOGENESIS IN HUMAN BRONCHIAL TISSUES

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The goals of this project are to use human lung tissue to study the development of preneoplastic (and possibly neoplastic) lesions induced by chemical carcinogens, and to study the ability of selected agents to modify the effects of chemical carcinogens on human lung tissue. Human bronchial epithelial tissues are to be exposed in vitro to chemical carcinogens, as well as to anti-carcinogens. These human bronchial tissues are then to be transplanted and maintained in vivo in the hamster and in the athymic nude mouse as xenografts.

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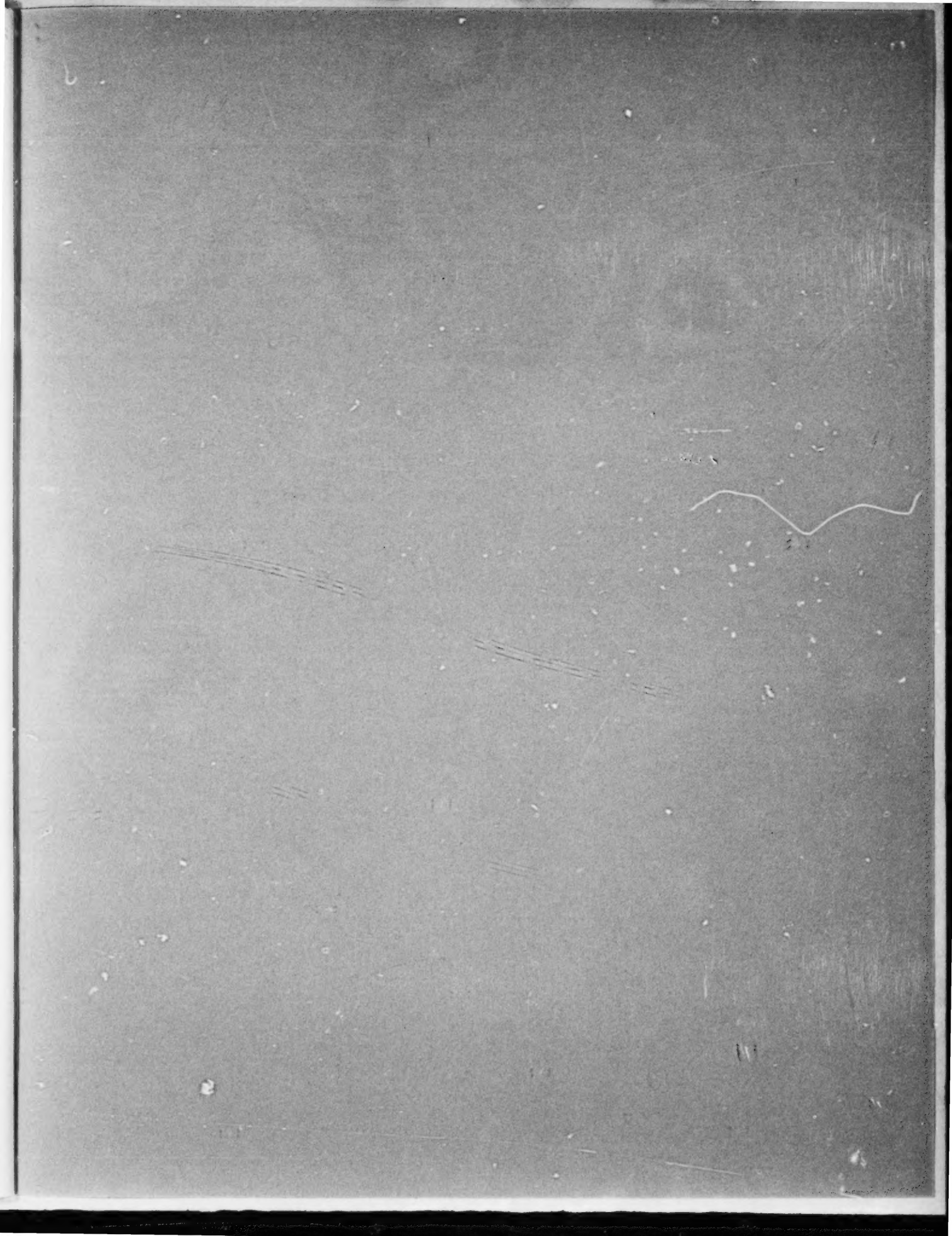

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